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Report No. 712437

EVALUATION OF BACTERIAL BINDING AND RELEASE OF CADMIUM FROM AQUATIC SEDIMENTS

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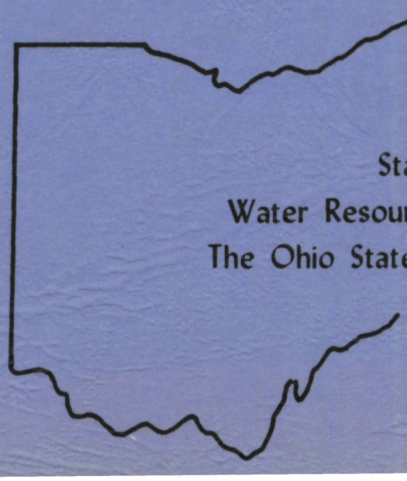
January, 1982

United States
Department of the Interior

Contract No.
B-079-OHIO



State of Ohio
Water Resources Center
The Ohio State University

EVALUATION OF BACTERIAL BINDING AND RELEASE OF CADMIUM FROM AQUATIC SEDIMENTS

by

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This study was supported in part by the
Office of Water Research and Technology
U.S. Department of the Interior under
Project B-079-OHIO

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INTRODUCTION

Elevated concentrations of heavy metals are a problem in the environment due to their toxicity to plants, animals, fish, and, ultimately, man. These metals may be concentrated in plants and passed throughout the food chain. The role of bacteria in the mercury cycle has been studied extensively, but how bacteria influence the movement of other metals such as cadmium is less well understood.

In man, the effects of cadmium were first identified after the occurrence of a malady called Itai-Itai (literally Ouch-Ouch) disease (Friberg et al., 1971). Acute effects included liver and kidney problems, due in part to high levels of Cd in these tissues (Bernstein et al., 1974) and skeletal decalcification (Friberg et al., 1971). Cadmium has been reported to be a human carcinogen (Loeb and Zakour, 1980) and cadmium oxide may cause an increased number of prostatic carcinomas in workmen exposed to CdO dust, especially when combined with smoking (Kolonel and Einkelstein, 1977). No sign of tetraogenic effects of Cd are known in man (Degraeve, 1981), although a possible weak correlation between Cd level in mother's blood and fetal blood has been reported, suggesting that the placenta can act as a barrier to Cd transfer (Lauwerys et al., 1978).

In other mammals various cadmium-induced effects have been described. Intramuscularly injected Cd caused sarcomas in rats (Heath et al., 1962) and an increased rate of resorption and malformed embryos in golden hamsters after cadmium sulfate treatment (Ferm and Carpenter, 1967). Cadmium has been reported to affect fertility in mole rats (Lee and Dixon, 1973) and to induce cellular and vascular changes in the ovaries of pre-puberal female rats (Kar et al., 1959).

Cadmium has also been reported to interact with the uptake and utilization of nutrients in laboratory animals. The toxicity of Cd was lessened by the presence of Cu, Fe, Mn, Se, Zn, and Vitamin C. Low Ca levels increased Cd toxicity as did pyridoxine and Pb. The synthesis of Vitamin D was inhibited by the presence of Cd (Sandstead, 1977).

In mammalian systems cadmium may induce the presence of metallothionein, a metal-binding protein (Kagi and Vallee, 1960). Metallothionein specifically binds Cd, Cu, Hg, and Zn (Shaikh and Lucis, 1972) and has been reported to be present in vertebrates (Kojima et al., 1976), invertebrates, and eukaryotic microorganisms (Olafson et al., 1979).

This study was undertaken to examine how cadmium may affect bacteria, both in laboratory microcosms and in natural sediment populations. Bacterial populations in a metal-contaminated river were examined and compared to a less contaminated site. Cadmium was added to a model aquatic system and the affects of the metal on bacterial populations in the sediment and water column were followed. The competition between bacteria and sediment for Cd in water was evaluated at different temperatures, pH values, and redox potentials to gain an understanding of how bacteria may be involved with uptake and subsequent mobilization of cadmium in the environment.

MATERIALS AND METHODS

Preparation of Cadmium Standard

Cadmium was prepared as a 1000 ug Cd(II)/ml solution by dissolving 1.631 g CdCl in 1 liter of double distilled demineralized water (ddH_2O). The solution was sterilized by filtering through a 0.45 µm membrane filter (Millipore Corp., Bedford, Mass.) and stored in a sterile glass bottle for not longer than one month before the preparation of a new stock solution. The actual Cd level was measured by comparison with a commercial standard Cd solution (Scientific Products, McGraw Park, Ill., Lot #8332). The solution was concentrated or diluted as needed to reach a final concentration of 1000 ug Cd(II)/ml.

Chemical and Bacteriological Analysis of River Sediments

Sampling Sites

The Ottawa River in Lima, Ohio, was chosen for this study as it is known to be contaminated by heavy metals at a point source (Figure 1). Two sampling sites were chosen; the first (Site #1) was at the Long Road bridge in Allen Co., Ohio, approximately 7 miles (11.3 km) downstream from the Vistron Corporation, an electroplating plant known to be a point source of heavy metals including Cd (Naymik, 1977). The second site (Site #2) was the heavy metal contaminated site and was located about 12 meters downstream from the effluent outflow of Vistron. Other samples were taken at 0.1 mile (0.16 km) increments starting at the outflow and at sites of approximately 3 mi (4.8 km), 4.2 mi (6.8 km), and 6 mi (9.7 km) downstream.

Collection of Samples

Ottawa River sediment was collected at monthly intervals for a one

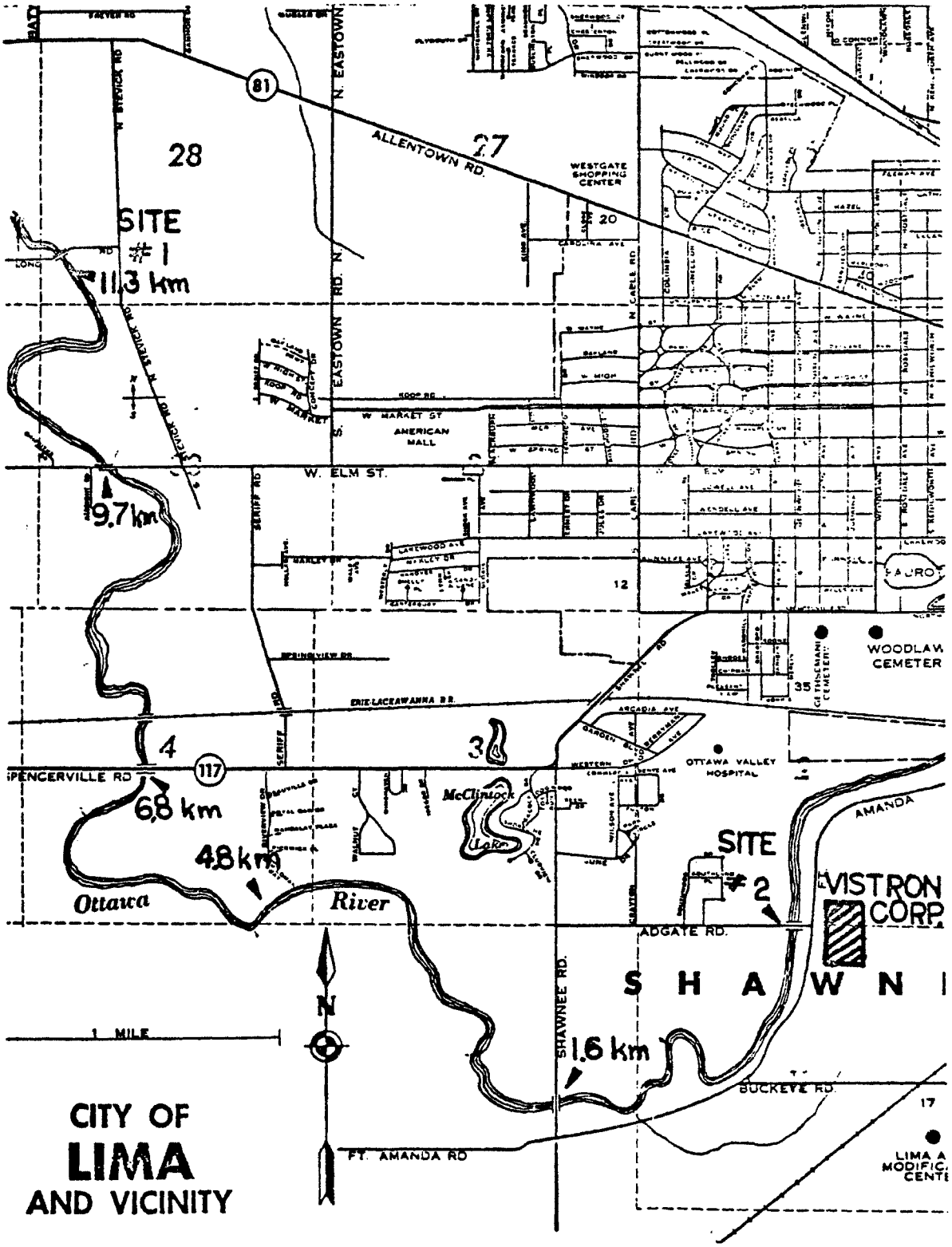

Figure 1. Map of Lima vicinity showing the downriver Site #1 and the metal-contaminated Site #2 at the outflow of the Vistron Corporation.

year period using a piston type corer made with a 50 ml syringe from which the tapered end was removed and tubular aluminum of the same outside diameter as the inside diameter of the syringe barrel (2.5 cm). The syringe barrel and rubber tip were autoclaved separately and assembled before collection of samples. Approximately 3 cm of sediment were collected with each insertion into the sediment. A sediment core was placed in each of two ca. 100 ml sterile wide mouth bottles containing 60 ml of diluent and glass beads (Anthony, 1970). The diluent was phosphate buffered dilution water (Bordner and Winter,1978). Several cores were also placed in plastic bags and in a third bottle. The bags were returned to the laboratory and frozen. The remaining bottle was used to measure Eh and pH of the sediment solution. The dilution bottles were shaken and serial dilutions were made immediately after retrieval using sterile diluent. Aliquots of 0.1 ml were spread on Plate Count Agar (Difco Laboratories, Detroit, Mich.) with or without 15 ug Cd(II)/ml. The inoculated plates were returned to the laboratory and incubated at ambient temperature ($23^{o} \pm 2^{o}C$) in the dark for 7 d before enumeration. Samples were given identification codes based on the number of sampling trips made to the river and on the site from which the sample was taken. Thus, the July 1, 1980 sample from the contaminated site was identified as 09-2, and the August 1, 1980 sample from the downstream site was labeled 10-1.

Analysis of River Sediment Samples

Frozen sediment samples were dried at $65^{o} \pm 2^{o}$ C and pulverized to pass through a 60 mesh sieve. Samples thus prepared were placed in Whirl-pak (Nasco, Fort Atkinson, Wisc.) bags and stored in the freezer.

Metal analysis was carried out by placing 3-4 g sediment in a 150 ml

beaker, adding 12 ml concentrated HNO_3, and digesting at $100^{o} \pm 5^{o}C$ for 3 h with a watch glass cover (Kemp, 1971). After cooling, about 10 ml ddH_2O were added, and the digests filtered (Whattman #44, Clifton, New Jersey). The digests were made up to 25 ml with ddH_2O. Metal content was analyzed by AAS using flame aspiration and standard conditions (see Appendix A).

Cation exchange capacity was performed by saturation with Na^+ followed by release with NH_4^+ and subsequent Na^+ measurement by AAS. The percent organic matter was analyzed by the Walkely-Black method which uses titration of potassium dichromate treated sediments with ferrous ammonium sulfate. Both procedures were done as outlined by Jackson (1958).

Isolation and Identification of Bacteria

Random colonies were picked from PCA plates after enumeration of October samples (trip number 12), streaked onto fresh PCA and grown at 25 C for one week. Bacteria were selected from plates both with and without the addition of 15 ug Cd(II)/ml and from both sampling sites. Approximately 50-60 isolates were picked from each parameter for the original isolation. The selected bacterial strains were restreaked onto PCA a second time, and then the purified organisms were put on two PCA slants and assigned an individual isolate number. One slant was used for storage of the bacteria at -70 C by the method of Feltham et al. (1978), and the other was used as the stock slant for identification.

The isolates were identified to the generic level using routine microbiological methods, including direct observations of colonies, microscopic observation, and biochemical analysis. Table 1 shows the identification scheme followed. Flagellar arrangement was examined by placing unfixed motile bacteria (as determined by wet mounts and light

TABLE 1

IDENTIFICATION SCHEME FOR BACTERIA ISOLATES

Culture ID # _____	Date isolated _____
Gram reaction _____	Cell shape _____
Arrangement _____	Colony shape _____
Motility _____	Flagellation _____
Catalase _____	Oxidase _____
Nondiffusible pigment _____	Diffusible pigment _____
Spore _____	H_2S _____
Indol _____	Citrate _____
MR _____	VP _____
Russell Double Sugar butt _____	Nitrate $NO_3^- \rightarrow NO_2^-$ _____
slant _____	$NO_3^- \rightarrow N_2$ _____

microscopy) on Formvar-carbon coated electron microscope grids, shadowing with germanium, and viewing with a Phillips EM-300 electron microscope. The bacteria were identified with the aid of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) and the Gram-negative identification scheme of Shewan et al. (1960).

Determination of Levels of Cd Resistance among Isolates

Bacterial isolates were grown in Brain Heart Infusion (Difco) and 50 µl was spotted onto the surface of PCA slants in 13 x 100 mm tubes. The agar medium was amended with additions of 0, 1, 5, 10, 20, 30, and 50 µg Cd(II)/ml. The tubes were incubated at ambient lab temperature and were scored for the presence or absence of growth on the different Cd concentrations after one week.

Model Aquatic Systems

Two model aquatic systems in 10 gallon aquaria were assembled in the laboratory in a manner similar to that described by Titus et al. (1980). The systems were arranged as shown in Figure 2.

Aquaria Components

Sediments. The sediments were stratified as shown in Figure 3. White sand (average diameter 0.45 mm), aquarium gravel (average diameter 1.3 mm), and aquarium pebbles (average diameter 5.5 mm) were purchased from Byerley's Aquarium Supplies (Columbus, Ohio). Potting soil was a product of Stim-U-Plant Laboratories, Inc. (Columbus, Ohio). Olentangy River mud was collected from a site approximately 50 meters south of the Drake Union on the Columbus campus of The Ohio State University. Mud was introduced into the tanks immediately after collection and served as the source of diversified microorganisms. Sand, gravel, and pebbles were washed in

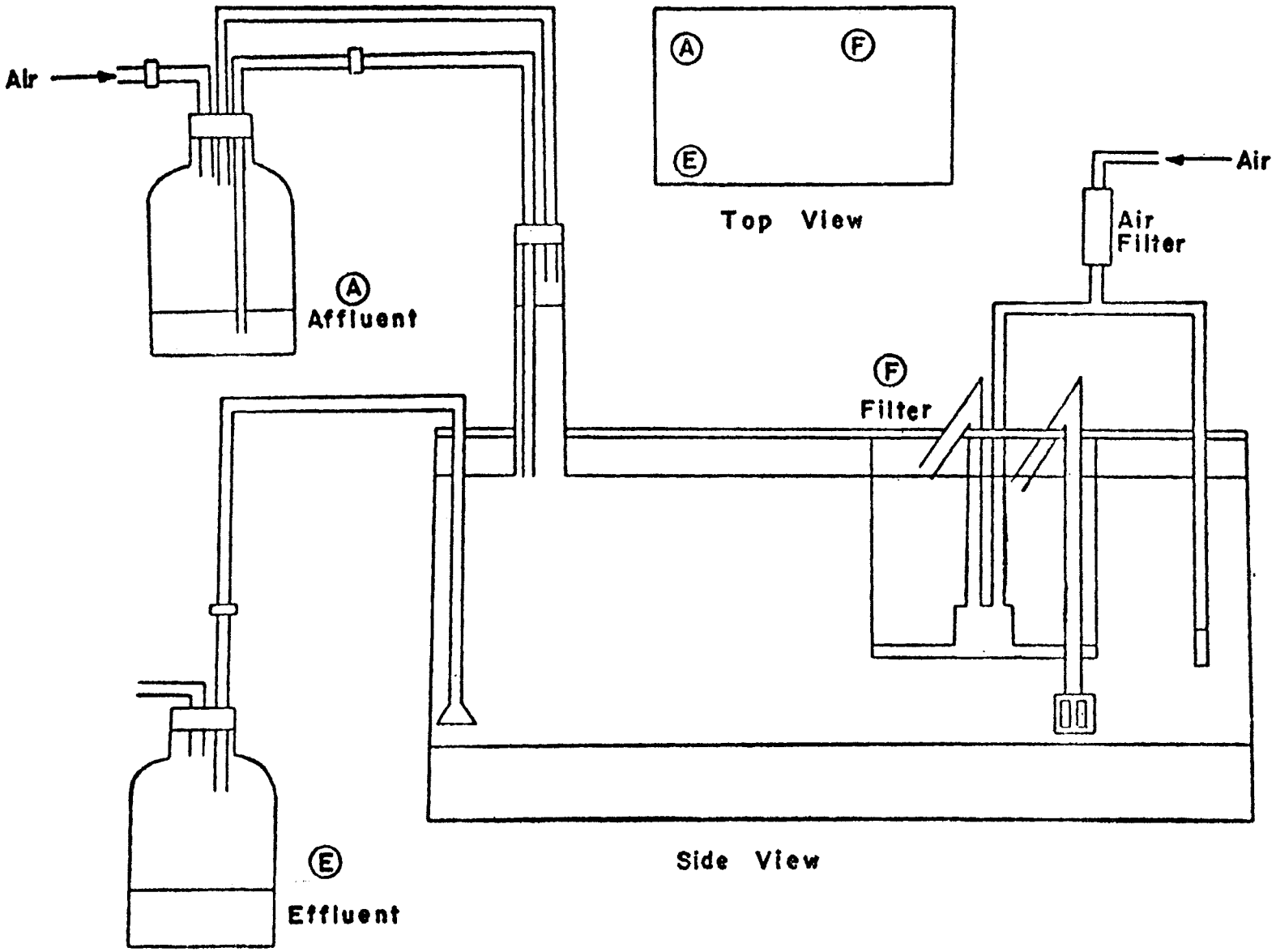

Figure 2. Design of model aquatic system.

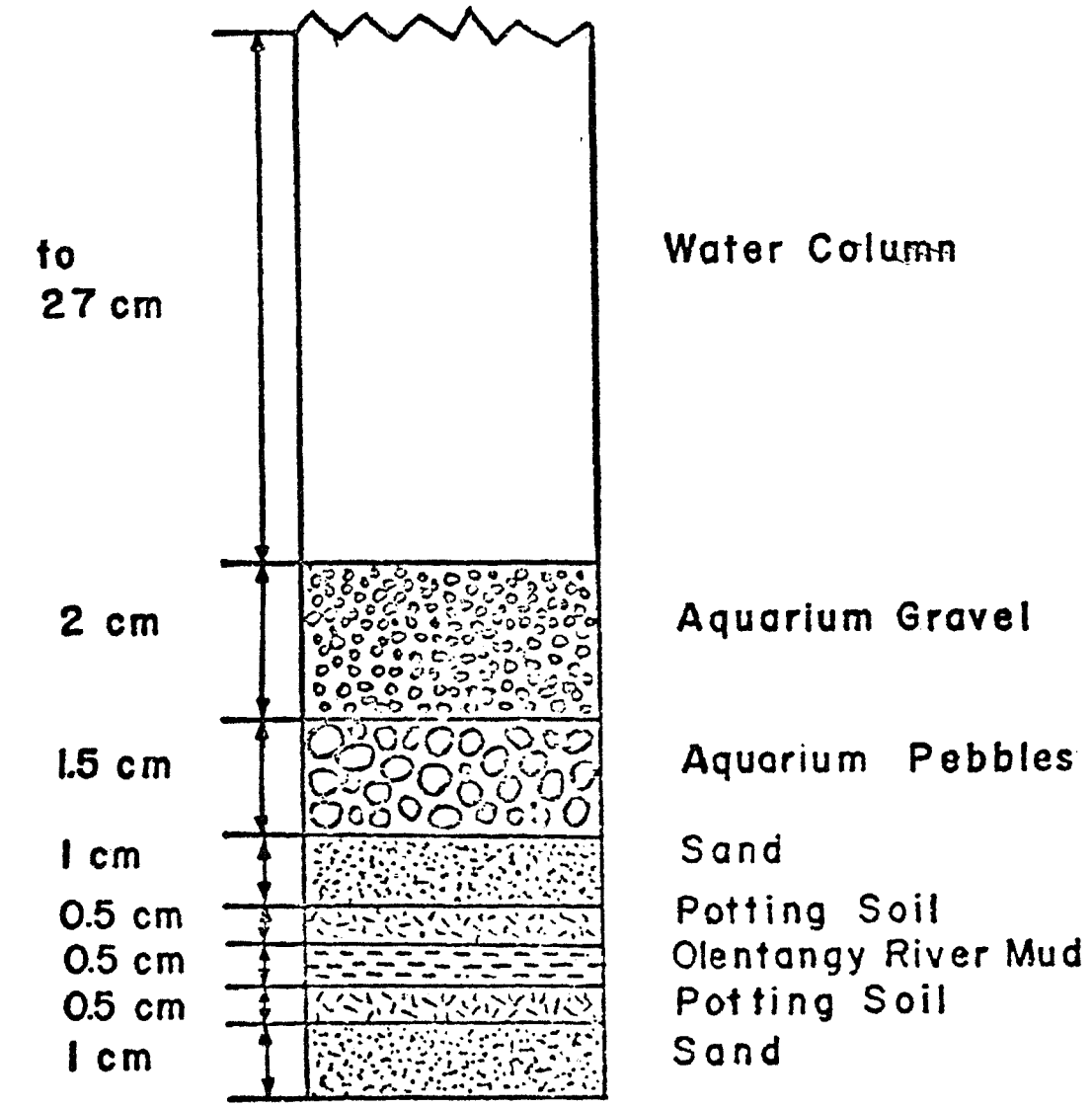

Figure 3. Stratification bed sediments of the model aquatic system

distilled water and dried prior to use.

Water. Demineralized distilled water was added and maintained at a level of 27 cm above the bottom of the aquaria and at a flow rate of 2 liters per day. This rate resulted in a residence time of about 12.5 days.

Filtration. Side filters containing Filter Floss (Finney Products, Inc., Cincinnati, Ohio) were used to clarify the water and were cleaned as needed.

Illumination. Light was supplied by fluorescent bulbs (15 watt, Sylvania-Enhance) which were mounted in the aquaria covers at a distance of 6 cm above the water surface.

Aeration. Air (1500 cc/min) was supplied by first passing laboratory bench compressed air through a cotton filter and then fritted glass spargers.

Regulated cadmium flow. After an equilibrium period of one month, 2.0 liters of fresh water either with or without 5.0 mg Cd(II)/l (10.0 mg Cd(II)/d) were added to the incoming water containers daily.

Analysis of Components

Weekly sediment samples (mainly Olentangy River mud and potting soil) were removed from both tanks and either dried at $65^{o} \pm 2^{o}C$ for cadmium analysis or placed in dilution bottles for bacterial analysis. Dried sediments were pulverized and passed through a 60 mesh sieve. Approximately 0.05 to 0.10 g were placed in 1.5 ml polypropylene (Bio-Rad Laboratories, Richmond, Calif.) test tubes, and 400 µl of concentrated HNO were added. The tubes were placed in a clamping device, heated at $50^{o} \pm 1^{o}$ C for 3 h, diluted with 800 µl of double distilled water, and centrifuged

for 3 min (Eppendorf Model 5412, Brinkman Instruments, Inc., Westbury, New York). The supernatant was removed, diluted further if necessary, and analyzed by AAS using the flame microsampling method similar to that outlined by Voth (1981). Standard additions (see Appendix A) of known quantities of Cd in 50 µl ddH_2O were added to 50 µl of the sample digest in a 1.5 ml test tube, and the combination was aspirated into the flame of the AAS to which a fast responding recorder (Kipp and Zonen, Delft, The Netherlands) was connected.

The cadmium level in the water column was analyzed by removing portions of the water from each tank and aspirating them, either directly or following dilution, into the flame of the AAS.

Bacteria in aquaria sediments and water were enumerated in the same manner as for river sediments. Individual isolates from the plates containing 15 µg Cd(II)/ml were identified to the generic level as previously described.

Adsorption of Cadmium by Bacteria and Sediments

Sorption of cadmium from water by bacteria and sediments was based on the methods outlined by Ramamoorthy et al. (1977) and Laube et al. (1979) for mercury, cadmium, and copper uptake studies. The system was contained in one liter reaction kettles (Pyrex #6947, Corning Glass Works, Corning, New York) which had covers with four openings and consisted of water, bacteria, and sediment components (described separately below) that were aseptically added to the sterile kettles. The system was incubated up to 4 d at pH values of 6, 7, or 8.5, temperatures of 4^{o}, 23^{o}, and $35^{o}C$, and at high (air-equilibrated) and low Eh values. The water in each kettle was stirred with a teflon-coated stir bar.

Four kettles were assembled for each parameter studied: water only, water and bacteria; water and sediment; and water, bacteria, and sediment. The standard conditions used for comparison of the test parameters were 23° C, ph 7, and air-equilibrated. Tests involving pH changes were done at 23° C and air-equilibrated, and those experiments involving temperature effects were carried out at pH 7 and air-equilibrated. The study at a lowered redox potential was done at pH 7 and 23°C for 2 d.

The Eh value of the system was initially lowered to 30 mV by purging the water of the sealed kettles with filtered nitrogen gas for 30 min at 8 h intervals. The Eh was measured with platinum combination electrodes (Fisher Scientific Co., Pittsburgh, Pa.) which were sterilized by immersion overnight in Chlorox solution.

Bacterial Component

The Pseudomonas sp. (isolate #12-2/15/21) used in these experiments was isolated from Ottawa River sediments and was resistant to 50 ug Cd(II)/ml on PCA. The bacterium was grown in Nutrient Broth (Difco) to late log-phase, harvested by centrifugation at 6,000 x g for 20 min, and washed twice with phosphate-buffered saline (0.3 g/l KH_2PO_4, 0.6 g/l Na_2HPO_4, 8.5 g/l NaCl). Phosphate-buffered saline was added to a final level of approximately 10 mg dry cells per ml, and 1.5 ml was injected into sterile dialysis tubing tied closed with nylon monofilament line. The remaining cells were washed once with ddH_2O and dried at 100° ± 5° C to determine the dry weight of cells used.

Sediment Component

Artificial sediments consisted of 1 part of Potting Soil and 3.5 parts of acid washed Kaolin clay (American Standard, Fisher Scientific

Co., Fairlawn, New Jersey). Potting soil was pulverized to pass through a 60 mesh screen. The artificial sediments had a cation exchange capacity of 30.4 mEq/100 g and consisted of 12.9% organic matter. Sediments were placed in dialysis bags (0.3 g sediment in 1.5 ml ddH_2O) and autoclaved suspended in ddH_2O.

Water Component

The water component of the system was a phosphate-carbonate buffer system (Sharp et al., 1980) with added salts. The composition of the buffering systems at different pH values is shown in Table 2. Components were autoclaved separately and then made up to volume after cooling. The buffer needed for each group of kettles (4 l) was made up in one flask, cadmium was added (1 ug Cd(II)/ml, nominal), and the system was allowed to equilibrate for one day. One liter of buffer was added to each kettle, bacteria and sediments in dialysis bags were added to the proper kettles, and samples were withdrawn after incubation for various intervals of time.

Analysis of Cadmium in Components

Cadmium in water was analyzed by direct aspiration into the flame of the AAS. Sediment was centrifuged, dried at $65^{\circ} \pm 5^{\circ}C$, and pulverized. Cadmium in the artificial sediment was analyzed by the flame microsample method previously described for sediments of the model aquatic systems. Bacterial samples were diluted 1:1 with concentrated HNO_3 and digested for 3 h at $50^{\circ} \pm 1^{\circ}C$. The digests were then analyzed for Cd content by the flame microsampling technique.

TABLE 2

COMPOSITION OF PHOSPHATE-CARBONATE BUFFERING SYSTEM AT DIFFERENT pH VALUES

	g/l		
	pH 6	pH 7	pH 8.5
KH_2PO_4	3.50	1.30	0.91
Na_2CO_3	0.10	0.34	0.75
$MgSO_4 \cdot 7H_2O$	0.04	0.04	0.04
$CaCl_2 \cdot 2H_2O$	0.03	0.03	0.03
$NaNO_3$	0.025	0.025	0.025

RESULTS

Metal Levels and Bacterial Populations in the Ottawa River

The Ottawa River was chosen as a sampling site since it has been previously shown to be contaminated with metals (Naymik, 1977). Preliminary sampling trips showed the river to be most contaminated near the effluent outflow of the Vistron Corporation in Shawnee Township, Allen County, near Lima, Ohio (Appendix B). The initial sample showed that the sediment under the Adgate Road Bridge over the river was highly contaminated with metals, and this site was designated #2. A second site was designated #1 and was located approximately 11.3 km (7 miles) downstream. The sediment of this site had reduced levels of metals, especially of Cd, Cr, and Ni.

The results of one year of sediment metal samplings are shown in Table 3. Paired t-statistics showed that the levels of Cd, Cr, Cu, Ni, Zn, and Fe were significantly (99% confidence level) higher at Site #2 than at Site #1. The background sediment levels of these metals in the Maumee River Basin area were (HNO_3:HCl extracted): Cd, 0.098 µg/g; Cr, 0.941 µg/g; Cu, 3.49 µg/g; Ni, 5.72 µg/g; Pb, 3.87 µg/g; and Zn, 8.78 µg/g (Naymik, 1977).

The level of organic matter in the sediment of the two sites did not vary significantly (Table 3).

Based on the average levels of the metals analyzed (other than Pb and Fe), two groups can be made. Cadmium, copper, and nickel were grouped together since the levels of the metals were reduced by about 50% between the contaminated and downriver sites (Cd, 48%; Cu, 48%; and Ni, 59%). Chromium and zinc (reduced by 94% and 81%, respectively) were reduced to a

TABLE 3

METAL LEVELS IN DOWNRIVER (SITE #1) AND CONTAMINATED (SITE #2) SEDIMENTS OF THE OTTAWA RIVER

Sample	µg metal per g sediment							Percent Organic Matter
	Cd	Cr	Cu	Ni	Pb	Zn	Fe	
09-1	2.37	38.6	29.4	45.2	82.2	117.8	18104	2.6
09-2	5.12	243.5	100.8	159.9	225.1	708.8	30835	8.8
10-1	4.76	14.1	89.5	53.3	245.2	211.8	27574	12.8
10-2	7.41	1007.5	109.2	198.5	209.8	1443.2	32156	10.8
11-1	3.40	13.8	63.8	39.3	502.0	288.6	24242	10.7
11-2	6.01	293.2	94.5	152.7	235.0	801.0	25771	8.3
12-1	2.65	47.3	55.2	113.0	88.2	225.8	30710	6.1
12-2	5.91	624.7	81.3	149.3	190.4	884.0	34024	8.7
13-1	2.11	43.0	22.6	60.2	111.6	87.6	12972	6.9
13-2	6.30	1780.4	48.5	167.4	182.6	681.5	19894	11.1
14-1	2.26	49.2	23.2	21.9	93.6	96.4	15806	6.1
14-2	5.44	823.1	47.9	76.7	188.7	462.4	19571	7.6
15-1	7.51	29.0	28.9	57.8	131.0	117.8	16376	6.9
15-2	6.83	551.9	41.6	182.3	174.3	692.9	17109	6.7
16-1	2.63	43.0	20.5	77.7	89.1	112.9	14404	6.1
16-2	10.20	516.2	51.0	116.2	242.1	632.9	17081	7.0
17-1	2.54	37.7	25.3	11.8	134.6	63.6	15247	5.7
17-2	5.19	645.4	46.9	20.5	196.5	972.5	16667	6.1
18-1	3.87	31.7	16.2	107.1	93.1	46.6	15023	5.7
18-2	9.64	364.6	35.0	281.6	156.6	180.3	17625	6.8
19-1	5.96	22.1	11.2	151.2	77.8	28.3	12145	3.5
19-2	9.48	321.8	102.9	329.9	163.3	152.3	20064	8.6
20-1	3.57	47.8	21.3	54.5	193.3	112.5	13038	6.8
20-2	6.38	405.0	28.9	109.9	193.2	481.3	14738	5.0
$\bar{x}_1 \pm s_1$	3.64* ± 1.60	34.8* ± 12.1	33.9* ± 22.3	66.1* ± 38.4	153.5 ± 115.7	128.3* ± 78.1	17970* ± 5868	6.7 ± 2.6
$\bar{x}_2 \pm s_2$	6.99* ± 1.73	631.4* ± 408.3	65.7* ± 28.3	162.1* ± 79.9	196.5 ± 25.9	674.4* ± 334.4	22128* ± 6470	8.0 ± 1.7

* Significantly different at 99% confidence level

greater degree upon travelling downstream than were the other metals.

Analysis of bacterial populations at the two sampling sites showed that the downstream site (#1) had a significantly higher total viable count per gram (TVC/g) than did the metal contaminated site (#2; Fig. 4). Both sites showed increased numbers of organisms at the December (14-samples) and May (19-samples) samplings. The first samples also had high numbers which could be related to the May peak. This may be due to differences in water and weather conditions between the spring of 1980 and the spring of 1981. Cadmium-resistant bacteria (15 μg Cd(II)/ml in Plate Count Agar) made up a significantly higher percentage of the TVC at site #2 than at the less contaminated Site #1. At site #1 the average level of resistance was 15.4% and at Site #2 the average level was 23.5%. The level of resistance was highest during the winter months (January and February, 1981) when the water temperatures were the lowest (Appendix C).

Genera of isolated and identified bacteria are shown in Figure 5. Most of the isolates from both sites were oxidase-positive Gram-negative rods and Bacillus sp. Site #2 (12-1/00) had a significant number of Arthrobacter sp. The isolates identified as 12-1/00 or 12-2/00 were initially taken from plates which had not been amended with cadmium. Isolates made initially from plates amended with 15 μg Cd(II)/ml (12-1/15 and 12-2/15) were composed of the same types of organisms. In these sets of organisms the number of Pseudomonas sp. was higher than in the sets of isolates which came from plates having no Cd amendment. The number of Bacillus sp. in these isolates was reduced and several Citrobacter sp. were isolated from the Cd-amended plates, while none were isolated from unamended plates.

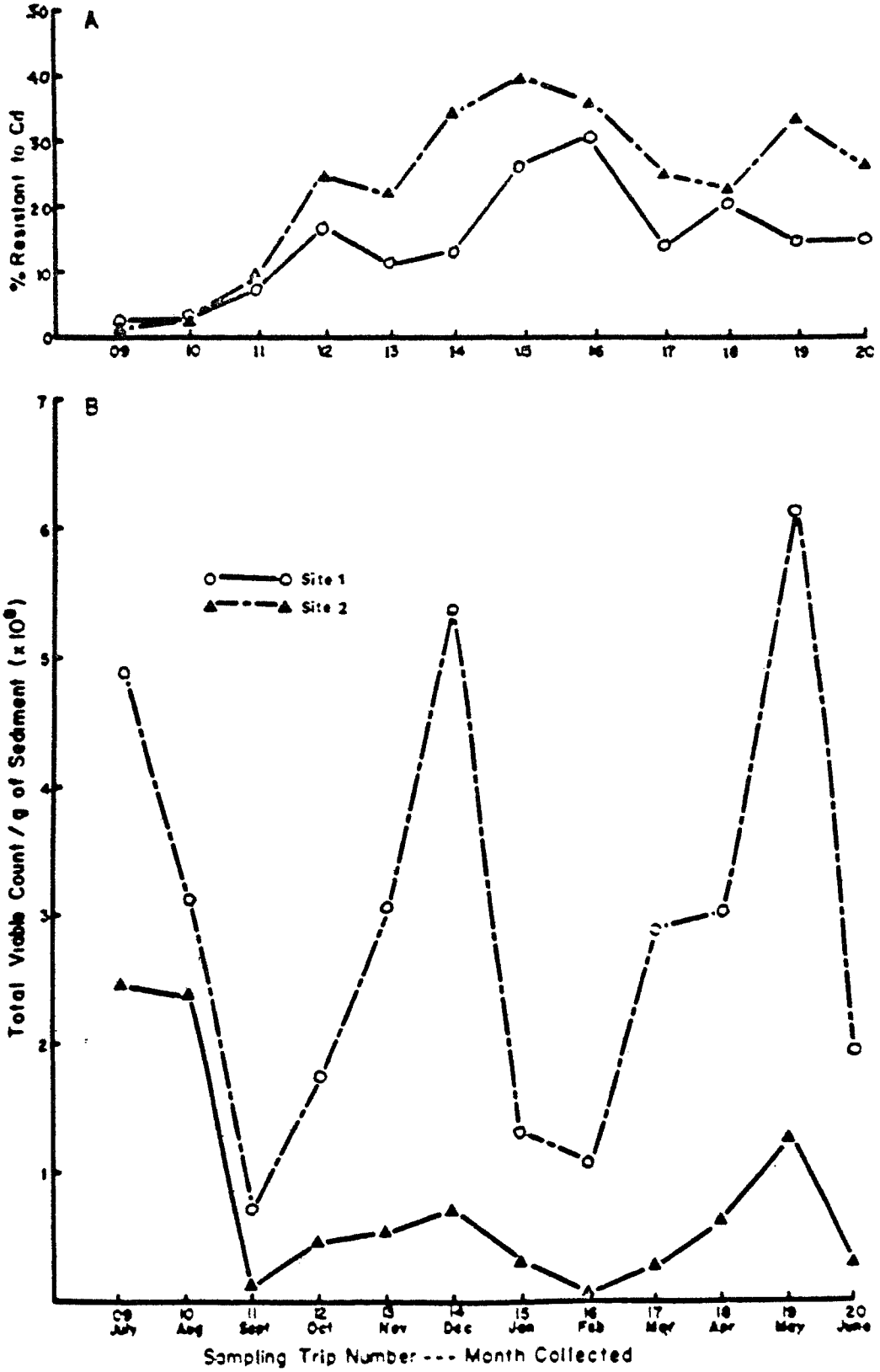

Figure 4. Total aerobic herotrophic bacterial counts (B) and the percentage thereof resistant to 15µg Cd^{2+}/ml (A) in the Ottawa River

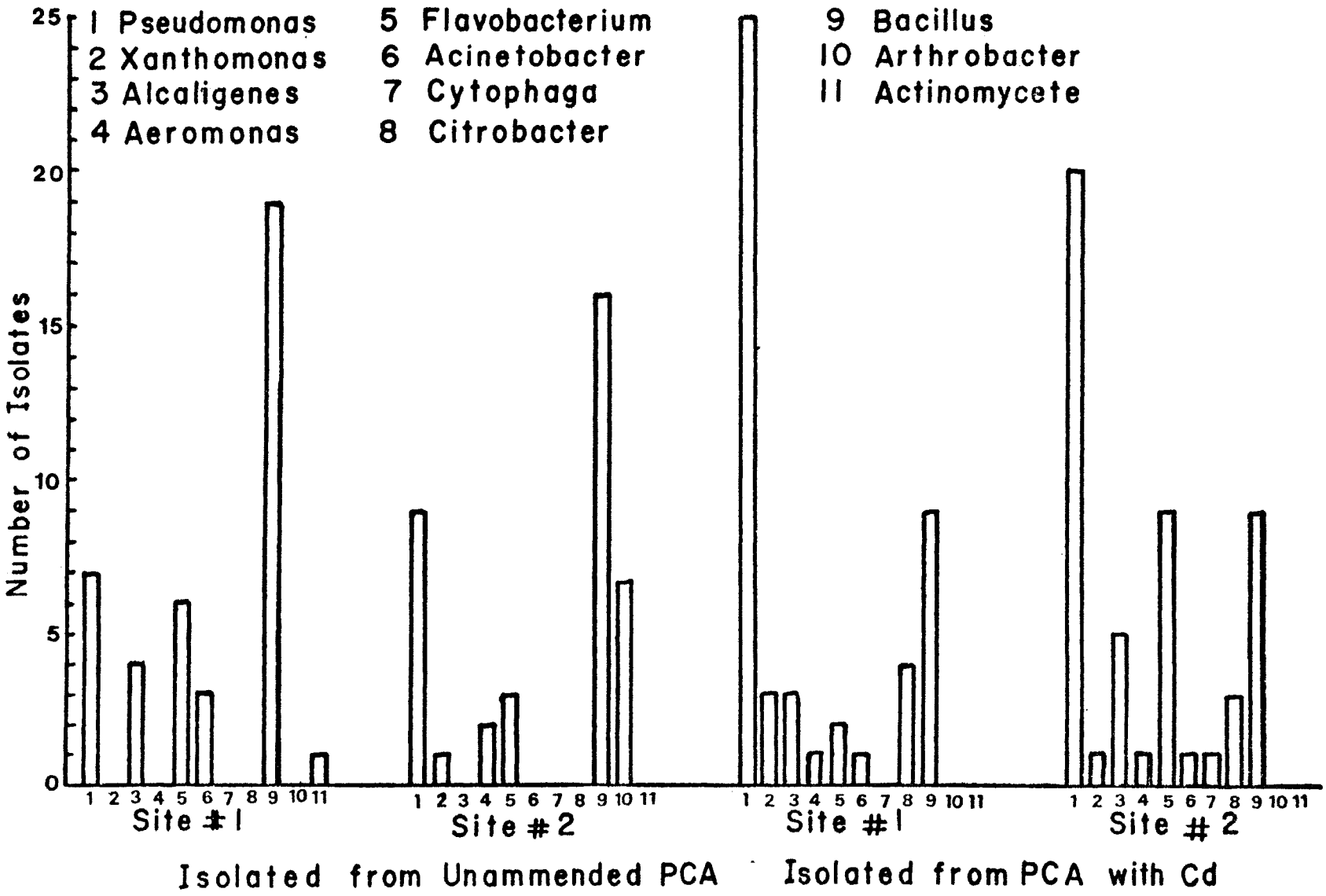

Figure 5. Genera of bacteria isolated from Ottawa River sediments from the October sampling trip (Trip #12)

Bacterial isolates were exposed to various concentrations of Cd, and the highest concentration which allowed growth was recorded. Table 4 shows resistance level vs. bacterial genera for the four categories previously described. Bacteria isolated from unamended PCA (12-1/00 and 12-2/00) showed more of a variance in the Cd-inhibitory level than did those from Cd-amended plates (12-1/15 and 12-2/15). The Bacillus sp. isolates showed Cd resistance from 0 to 50 μg Cd/ml in both categories from unamended plates. The resistance levels of Pseudomonas sp. were skewed toward higher concentrations of Cd in the 12-2/00 isolates (from contaminated Site #2) than in the 12-1/00 isolates (from the downriver site). In the 12-1/15 and 12-2/15 samples most of the isolates were resistant to at least 20 μg Cd/ml, but some showed a reversion from resistance to 15 ug Cd/ml (initial Cd level in PCA) to between 0 and 5 μg Cd/ml. Bacteria resistant to 10 μg Cd/ml may also be resistant to 15 μg Cd/ml, but not 20 μg Cd/ml, and therefore whether these isolates have lost some ability to resist the effects of Cd cannot be determined.

Distribution of Cd and Adaptation of Bacteria In the Model Aquatic System

Water Column

Addition of Cd(II) to the water of one of the model systems resulted in an increase in the Cd load in the water column (Fig. 6A). The cadmium level in the tank not receiving Cd remained below detectable levels ($<$ 0.01 μg Cd/ml) for the 10-week experiment. Total cadmium in the treated tank increased through the first two weeks, remained nearly constant through five weeks and then began rising again through Week 10.

The number of bacteria in the water column of the control tank

TABLE 4

NUMBER OF BACTERIAL ISOLATES (TRIP #12) RESISTANT TO VARIOUS LEVELS OF CADMIUM*

		Number of Isolates										
	Cd resistance Level ($\mu g\ Cd^{2+}/ml$)	Pseudomonas	Xanthomonas	Alcaligenes	Aeromonas	Flavobacterium	Acinetobacter	Cytophaga	Citrobacter	Bacillus	Arthrobacter	Actinomycetes	Number of Resistant Isolates
12-1/00	0	3				2	1			1			7
	1			1		2				4			7
Site #1	5						1			2			3
	10	3		2		2	1			3			11
No Cd	20									1			1
Initially	30			1						3			4
	50	1								4		1	6
12-2/00	0									3			3
	1	1			1	1				1	2		6
Site #2	5				1	1				1	2		5
	10	3	1			1				3			8
No Cd	20	2								3	2		7
Initially	30	2								3	1		6
	50	1								2			3
12-1/15	0												0
	1					1							1
Site #1	5												0
	10									2			2
Cd	20	4		1		1				7			13
Initially	30												0
	50	21	3	1			1		4				30
12-2/15	0	1											1
	1	1			1	1		1			1		5
Site #2	5												0
	10					1	1			3			5
Cd	20	2				1				1			4
Initially	30	3	1	1		1				3			9
	50	13		1		3			2	2			21

*12-1/00: Downriver site; isolated from Plate Count Agar (PCA) without Cd.
12-2/00: Contaminated site; isolated from PCA without Cd.
12-1/15: Downriver site; isolated from PCA amended with 15 g Cd(II)/ml.
12-2/15: Contaminated site; isolated from PCA amended with 15 µg Cd(III)ml. Cd(II)/ml.

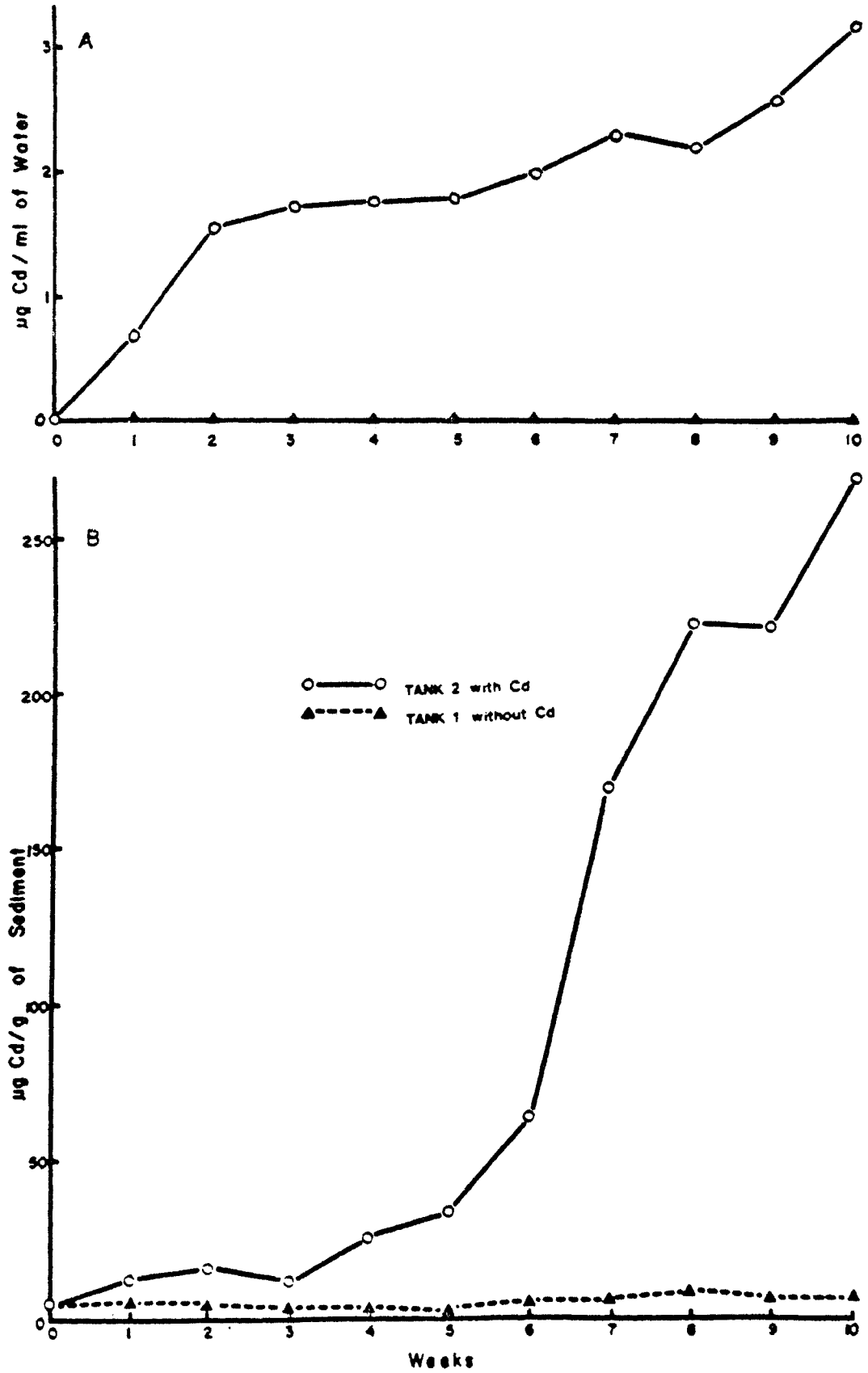

Figure 6. Levels of cadmium in the water columns (A) and sediments (B) of the model aquatic systems.

remained nearly constant through the 10 weeks (Fig. 7B). In the Cd-treated system there was an increase in the concentration of viable cells following cadmium addition. The percentage of the total count that was resistant to 15 ug Cd(II)/ml in the control tank fluctuated between 1% and 10% during the experiment, while the level of resistance of the bacterial population in the treated system showed an increase within the first week and continued to follow an increasing trend throughout the remainder of the experiment (Fig. 7A). The final resistance level found was 88% while the highest noted (Week 5) was 98%.

Sediment

Initial Cd levels in components of the model systems are shown in Table 5. Sediment Cd levels in the two model systems began at nearly the same concentration and the untreated system remained at or near the original concentration (Fig. 6B). The cadmium level in the tank that received the metal rose slowly through the first five weeks, and then it began to increase at a higher rate. A level about 50 times that of the initial Cd concentration was reached after ten weeks. The cadmium level was still increasing after ten weeks, but rate of sorption appeared to be slowing. Between Weeks 5 and 6 the rate of Cd sorption to the sediment increased and was paralleled by an increased rate of Cd addition to the water column (Fig. 7A).

Bacterial populations in the control tank sediment decreased through the first five weeks and then rose for the remainder of the test period (Fig. 8B). The population varied less than one log value (3.88×10^8 TVC/g high and 7.42×10 low) during the experiment. The bacterial population of the Cd-amended tank sediments varied relatively little

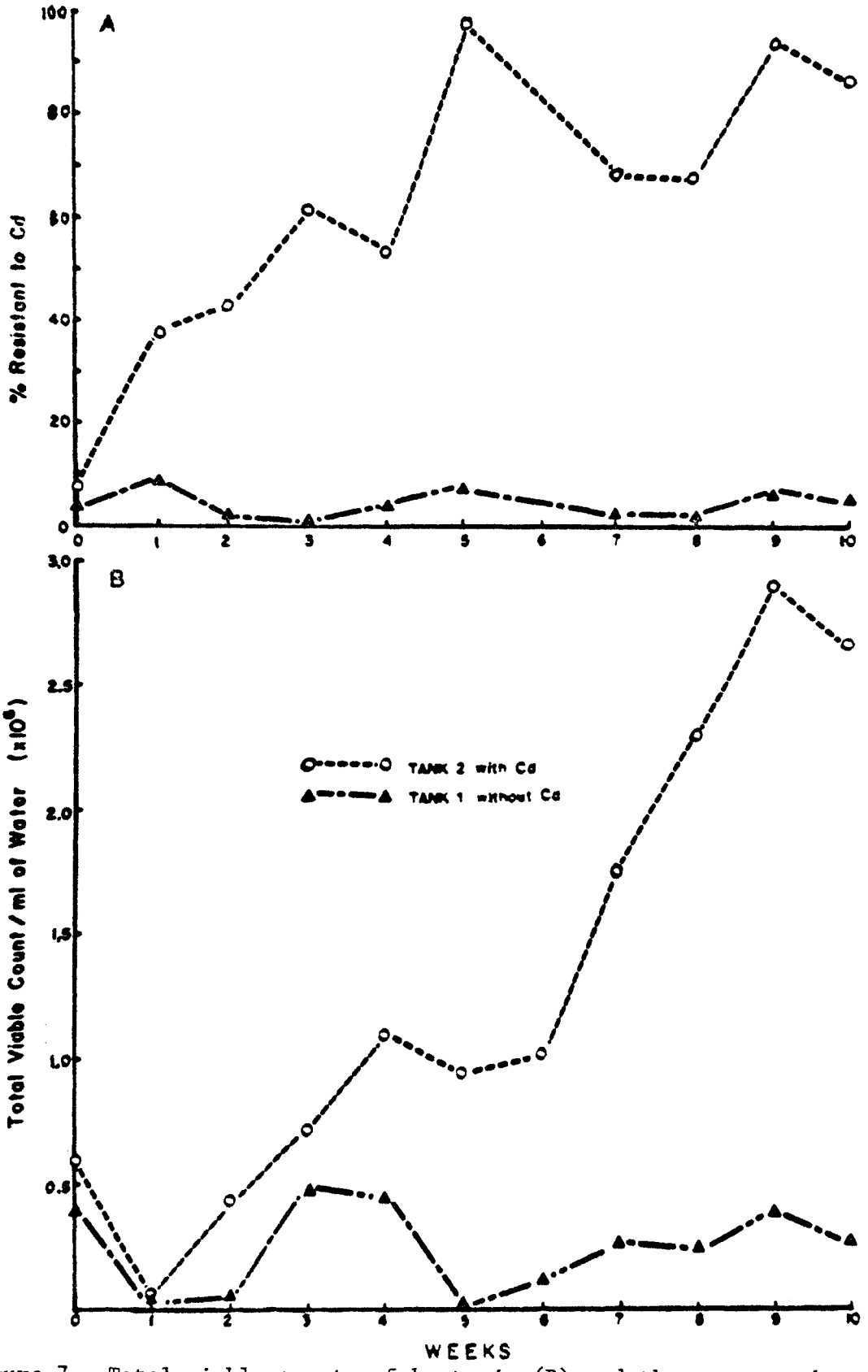

Figure 7. Total viable counts of bacteria (B) and the percent thereof resistant to Cd (A) in the water column of the model aquatic system

TABLE 5

CADMIUM LEVELS IN MODEL AQUATIC SYSTEM COMPONENTS

	µg Cd/gram
Olentangy River Mud	2.56
Potting Soil	0.64
White Sand	0.01
Gravel	0.01
Pebbles	0.01

through the first seven weeks and then reached a high at Week 8, followed by a decrease in numbers through Weeks 9 and 10. The number of viable bacteria at Week 10 was similar to the number prior to the large increase. The increase was observed during the accellerated rate of Cd sorption by the sediments (Fig. 6B), and the number of organisms in the sediment decreased even though Cd continued to be adsorbed by the sediment.

The level of Cd resistance by the sediment bacteria remained constant in both tanks for the first seven weeks (Fig. 8A), but the level of resistance in the Cd-amended tank increased at the same time as the TVC/g increased. Also, as the total number of bacteria in the sediment stabilized, the level of resistance bacteria returned to the normal level observed when Cd in the sediment was at a low concentration.

Bacteria were isolated and identified from PCA plates amended with 15 µg Cd(II)/ml before the addition of cadmium to Tank #2. Cd-resistant isolates were collected from the water column and the sediment of both tanks. After ten weeks another group of Cd-resistant isolates were collected and identified in the same manner as Week 0. The genera of organisms present in the highest numbers on the Cd containing plates from the model aquatic systems (Table 6) were similar to those found in Ottawa River sediments (Fig. 5). Cadmium-resistant Bacillus sp. were low in relative abundance in the water column, but made up a high percentage of those organisms identified from the sediment. Pseudomonas sp., Alcaligenes sp., and Flavobacterium sp. composed most of the organisms isolated from Cd-amended medium. The treatment of Tank #2 with Cd for 10 weeks had little effect on the relative abundance of the different genera in the sediment (0-T2S vs 10-T2S) or in the water column (0-T2W vs

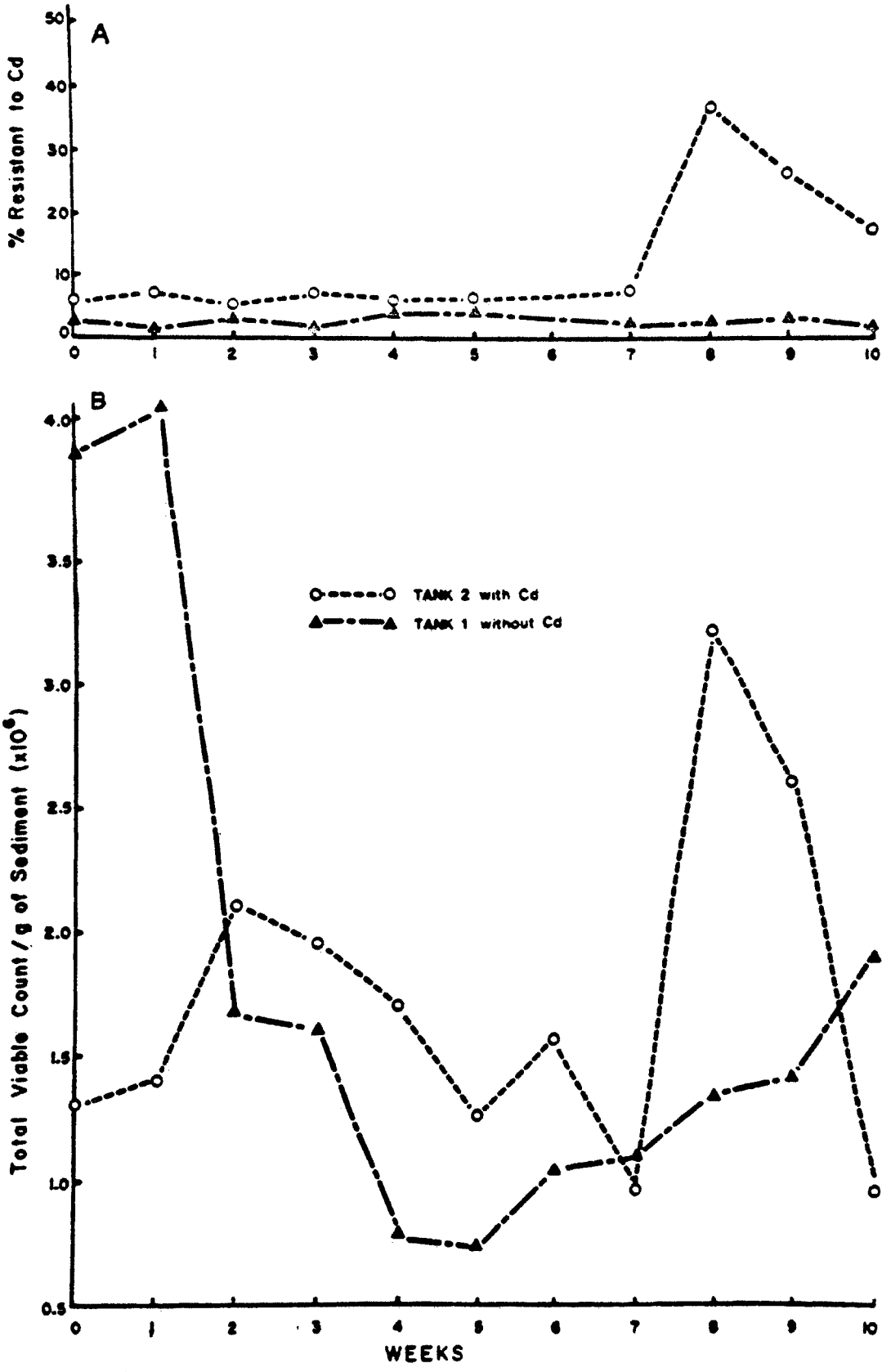

Figure 8. Total viable counts (TVC) of bacteria (B) and the percent of the TVC resistant to cadmium (A) in the sediment of the model aquatic system

TABLE 6

GENERA OF Cd-RESISTANT BACTERIAL ISOLATES FROM THE MODEL AQUATIC SYSTEMS BEFORE THE ADDITION OF CADMIUM (WEEK 0) AND TEN WEEKS AFTER CADMIUM ADDITION*

	Pseudomonas	Xanthomonas	Alcaligenes	Aeromonas	Flavobacterium	Acinetobacter	Cytophaga	Vibrio	Bacillus	Arthrobacter	Actinomycete
0-T1-S	9/23	1/23	3/23	0	1/23	0	0	0	9/23	0	0
(No Cd)											
10-T1-S	8/30	1/30	8/30	0	3/30	0	1/30	0	4/30	5/30	1/30
0-T2-S	7/22	1/22	1/22	0	6/22	0	0	0	6/22	0	1/22
(+ Cd)											
10-T2-S	5/20	1/20	1/20	0	4/20	1/20	0	1/20	5/20	1/20	1/20
0-T1-W	9/18	1/18	3/18	0	4/18	1/18	0	0	0	0	0
(No Cd)											
10-T1-W	3/18	1/18	6/18	0	1/18	1/18	1/18	2/18	3/18	0	0
0-T2-W	9/11	0	1/11	0	0	1/11	0	0	0	0	0
(+ Cd)											
10-T2-W	1/9	1/9	2/9	1/9	3/9	0	0	0	0	0	1/9

*0-T1S: Week 0; tank without Cd; sediment.
10-T1S: Week 10; tank without Cd; sediment.
0-T2S: Week 0; tank with Cd; sediment.
10-T2S: Week 10; tank with Cd; sediment.
0-T1W: Week 0; tank without Cd; water column.
10-T1W: Week 10; tank without Cd; water column.
0-T2W: Week 0; tank with Cd; water column.
10-T2W: Week 10; tank with Cd; water column.

10-T2W). The general change in the types of Cd-resistant organisms was apparently affected more by the aging of the model systems than by the Cd treatment since there is a shift toward increased heterogeneity in the genera isolated that were Cd-resistant.

Parameters Influencing Cd Adsorption By Bacteria and Sediment

The movement of cadmium in the environment may be influenced by various environmental factors which may exert effects on both the biological and physical portions of the ecosystem. Parameters investigated in this study included temperature, pH, and redox potential and their influence on Cd adsorption by bacteria and an artificial sediment matrix.

When a Pseudomonas sp. (Isolate #12-2/15-21) was placed in dialysis tubing and incubated in a phosphate-carbonate buffer system with 1 µg Cd(II)/ml, the bacteria adsorbed Cd at rates and quantities which varied with the incubation conditions. The standard conditions to which other treatments were compared was pH 7 - 23°C, and air-equilibrated (about 280-300 vV). The adsorption of Cd by bacteria at pH 7 - 23°C was approximately constant throughout the 4 d incubation (Fig. 9A). The rate of Cd adsorption at pH 6 - 23°C was initially the fastest of the treatments examined, but pH 7 - 35°C reached the highest final Cd concentration in the water-bacteria system. Cadmium adsorption was the lowest at pH 8.5 - 23°C, and adsorption at pH 7 - 4°C was less than pH 7 - 23°C. Lowering the Eh value (30 mV initially and 90 mV after 2 d) resulted in a 2 d Cd level midway between that of pH 7 - 23°C and pH 7 - 35°C. The final levels of Cd reached in bacteria in the water-bacteria

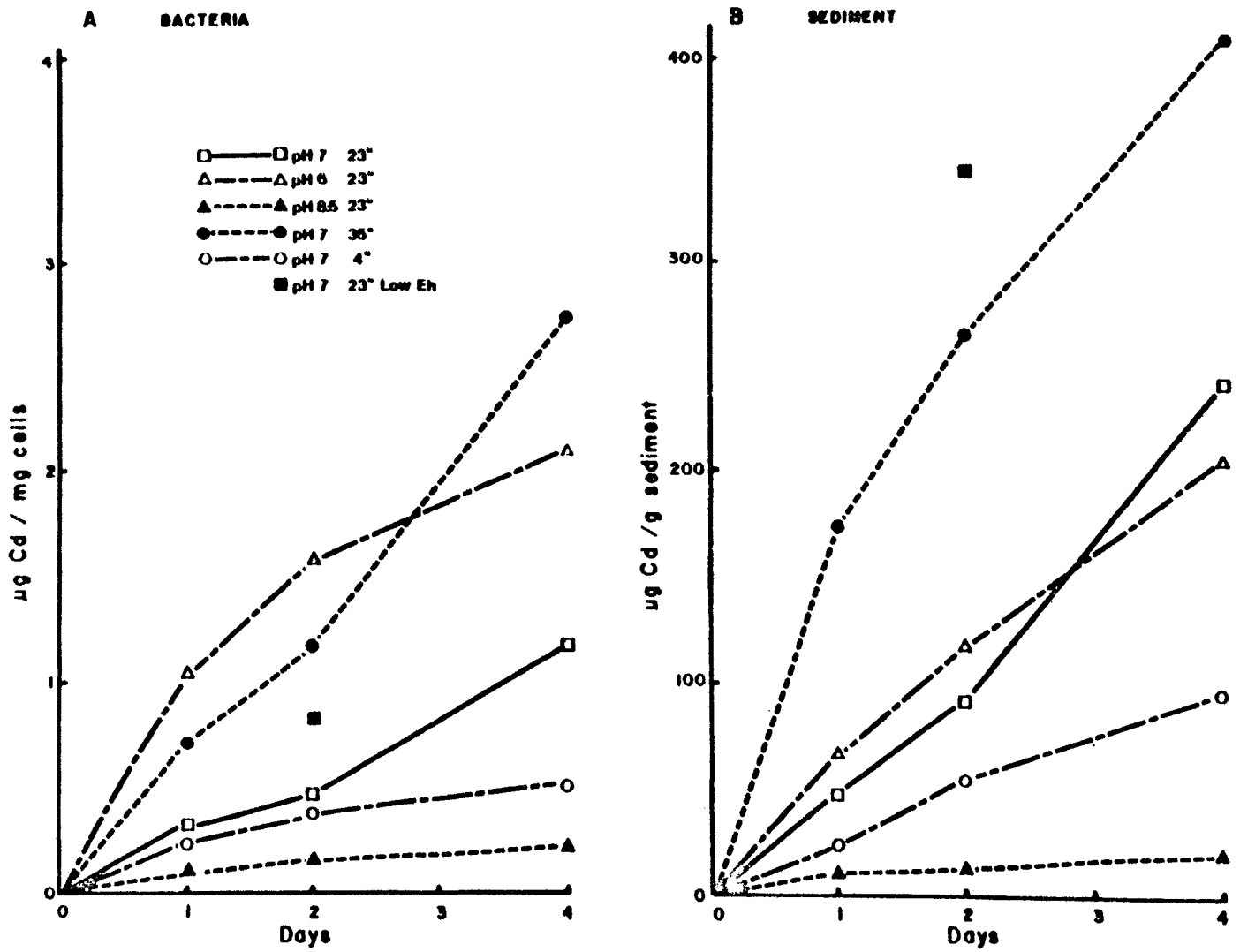

Figure 9. Uptake of cadmium by Pseudomonas sp. (A) and artificial sediments (B) under differing conditions when incubated in separate enclosures

system were measured in ug Cd per mg of bacterial cells (dry weight) and was in the range of 1 to 3 μg Cd/mg for the more efficient treatments.

Adsorption of Cd by the artificial sediment matrix in a water-sediment system was related to the incubation conditions in a manner similar to bacteria (Fig. 9B). Incubation at pH 7 - 23° C resulted in a relatively constant rate of Cd adsorption as did pH 6 - 23°C, pH 7 - 4°C, and pH 8.5 - 23°C. The initial rate of Cd adsorption at pH 7 - 35°C was the highest of the pH-temperature treatments and the final level of Cd reached after 4 d was also the highest. Cadmium levels reached after 4 d at pH 6 - 23 C and pH 7 - 23°C were similar, and pH 7 - 4°C and pH 8.5 - 23°C were lower than the standard in that order. Lowering the Eh value resulted in a higher 2 d Cd level than any of the other treatments.

Comparison of the water-bacteria-sediment systems (Fig. 10) with the water-bacteria and water-sediment systems showed that there was little difference in the final levels of Cd reached in bacteria or sediment or in the rates of adsorption between methods of exposure to Cd. The pH 6 - 23° C and pH 7 - 35°C treatments in the bacterial samples were reversed, and the pH 7 -23°C and pH 6 - 23° C incubations were reversed in the sediment when bacteria and sediment were incubated together. In both systems the rate of Cd adsorption by the Pseudomonas sp. was higher initially at pH 6, and then the rate slowed between 1 and 4 d. Cadmium was adsorbed at a more constant rate at pH 7 and pH 8.5 throughout the 4 d incubation periods (Figs. 9A and 10A).

The amount of cadmium adsorbed by bacteria averaged about ten times higher than Cd adsorbed by the sediment when based on dry weight. The difference was most pronounced during the initial day of Cd exposure and then lessened after 4 d in the presence of Cd.

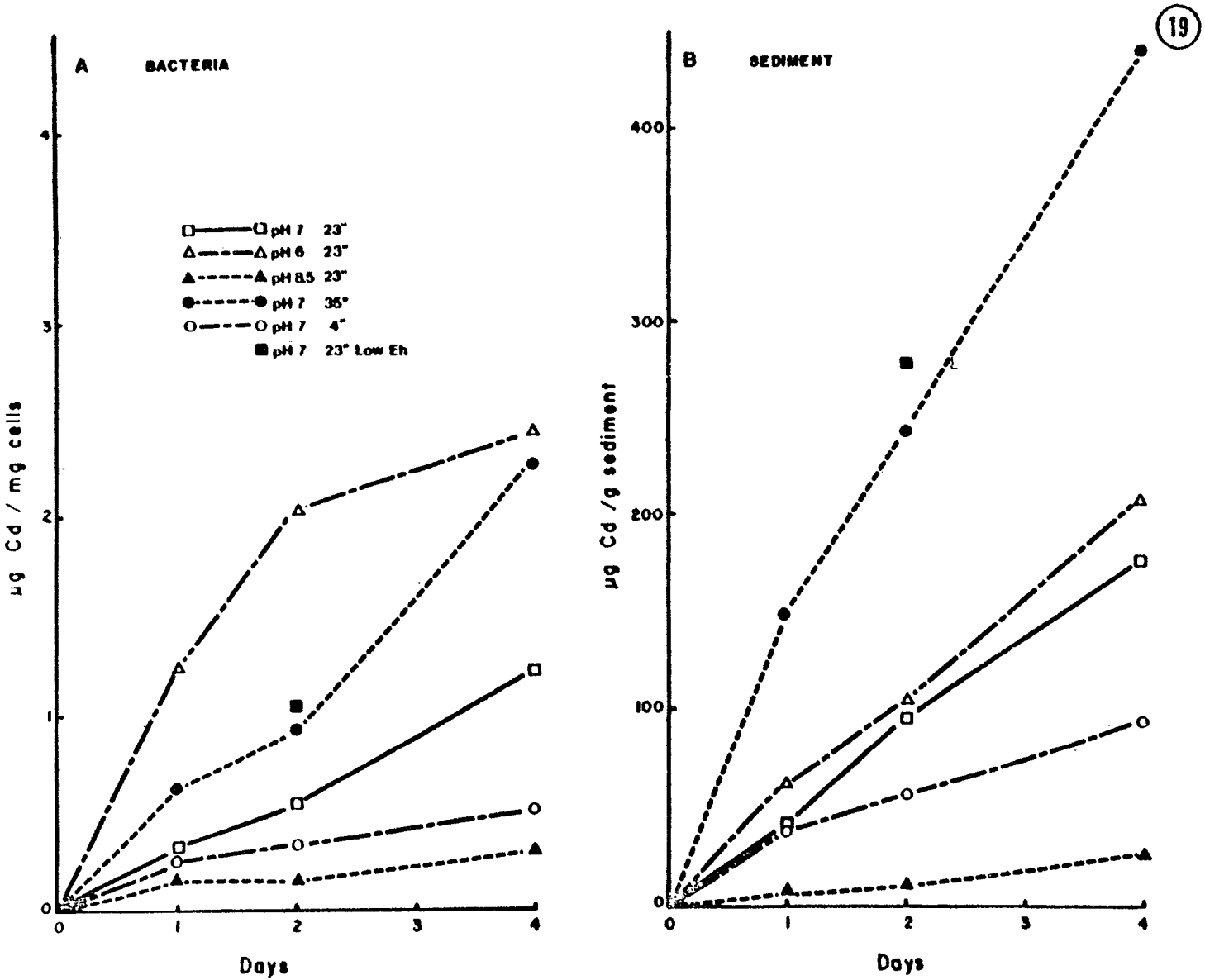

Figure 10. Uptake of cadmium by Pseudomonas sp. (A) and artificial sediments (B) under differing conditions when incubated in the same enclosures

DISCUSSION

The Ottawa River in Lima, Ohio, was shown to be contaminated at the site of the outflow from an electroplating plate. Levels of cadmium, chromium, copper, nickel, and zinc were shown to be elevated when compared with a site approximately 11.3 km downstream, while lead was not enriched near the outflow. Various factors could be responsible for the decline in the metal concentrations between the two sites. Generally, a high percentage of metal in the water column is bound to particulate matter which may settle out of the water column and add the metals to the sediment. The direct adsorption of the metals from the water column by the sediment may also occur.

Other factors are also responsible for the transport of metals in this river. The current in the river is associated with the water flow and, therefore, the season. In the spring the river can get over 2.4 meters deep at the contaminated site and about 1.2 m at the downstream site, while during the low water conditions in late summer the levels were about 0.7 m and 0.3 m, respectively.

Plate counts obtained from both locations in the Ottawa River showed that the total numbers of detectable bacteria were different. The uncontaminated Site #1 consistently had a higher total viable count (TVC) than the metal contaminated Site #2. Peaks in the TVC varied throughout the 12 months of sampling with the highest numbers appearing in July, December, and May, and these peaks were found in the TVC of both sites. No explanation for these peaks could be ascertained since two were in the spring-summer, and one was in the fall-winter. The peak in July, 1980 (Sample #09), and May, 1981 (Sample #19), could be related since the

spring high water period of 1980 was in April, while in 1981 the high water period was delayed until May and June. The spring peak may have been related to agricultural runoff entering the river and providing nutrients to the bacterial populations.

Cadmium resistance was defined as the ability to grow on Plate Count Agar amended with 15 μg Cd(II)/ml and was determined by preliminary platings of bacteria in the sediments. It was found that nearly 50% of the bacteria were resistant to 10 μg Cd(II)/ml, while only about 10% were resistant to 15 μg/ml. Additions of 20 μg Cd(II)/ml resulted in about an 8% resistance level. Because there seemed to be a nonlinear response to increasing Cd levels, the point where the breaks occurred (15 μg/ml) was used. Some other Cd concentrations that have been used to define resistance to the metal were 10^{-4} M (11.2 μg/ml) (Tyencka and Zylinska, 1974), 11 μg/ml (Timoney et al., 1978), 50 μg/ml (Pickett and Dean, 1976), and 100 μg/ml (Houba and Remacle, 1980; Mills and Colwell, 1977; Mitra and Bernstein, 1978). The type of medium used will influence the resistance of a particular bacterium to cadmium.

The level of Cd-resistance by the sediment populations initially was low at both sites. At Site #2 (metal-contaminated) the peaks in resistance coincided with increases in the TVC except during the first sampling period when the level of resistance was low. In contrast the highest percent of the TVC resistant to Cd at Site #1 was during the periods when the TVC was low, not at the peaks of the TVC as at Site #2. The significance of this observation is not clear, but it may be related to the types of organisms which could grow rapidly at a particular site. In the downstream sediments the ability to reproduce and survive may not

have been related to the concentration of metals present, while at the contaminated site the selective pressures exerted by metals (cadmium in particular) may have only allowed the more resistant strains to grow successfully. This would have led to an increase in the number of resistant bacteria detected.

The types of organisms isolated from both sites were similar and Bacillus sp. composed the highest percentage of the populations. The percentages of the isolated (not initially exposed to Cd) bacteria which belonged to the genera Pseudomonas and Arthrobacter were higher at the outflow of the electroplating plant than downstream. When the isolates were taken initially from plates amended with Cd, the highest percentages of bacteria isolated were Pseudomonas sp. and the percent of Bacillus sp. was reduced. The presence of these two genera has been reported in other studies of metal-contaminated environments and metal-resistant bacteria (Nelson and Colwell, 1975; Timoney et al, 1978). These studies were carried out in sea water environments, but the genera of bacteria reported by Nelson and Colwell (1975) corresponded closely to those found here. Houba and Remacle (1980) found that 100% of the Cd-resistant (100 ppm) bacteria isolated from a zinc-copper factory pond were oxidase and catalase positive and most were motile. Their analysis showed that 90% of these strains produced an alkaline reaction on mannitol and were proteolytic, while 77% of those strains which were Cd-resistant could oxidize sucrose, maltose, and glucose and had lipolytic capacities.

This study examined only aerobic, heterotrophic bacteria which were capable of growing on Plate Count Agar. No attempt was made to enumerate anaerobic populations. The reduced nature of the sediments would suggest

that the facultative anaerobes and anaerobes may make up a more significant portion of the total heterotrophic population than was found by aerobic incubation.

The level of Cd resistance in the bacteria isolated from the sediments showed that most of the genera reached high levels of Cd tolerance. Bacillus sp. and Pseudomonas sp. from the plates without Cd showed a large variation in their sensitivity to Cd. Isolates from Cd-amended plates maintained high resistance levels even when not grown in the presence of Cd for several transfers during isolation and purification. However, after three to four transfers several of these isolates lost the tolerance to 15 ug Cd(II)/ml which they possessed upon initial growth on PCA. The highest level of cadmium tested was 50 ug Cd(II)/ml, but many of the isolates may have been resistant to higher concentrations of the metal. A problem related to higher concentrations is that in complex organic media or in media with high levels of phosphates, Cd may precipitate with medium components, resulting in misleading levels of resistance being observed.

In the model aquatic system cadmium was found to enter the water column and sediment at different rates. The water Cd concentration initially rose rapidly and then increased little after about three weeks. During this period the level of Cd in the experimental tank increased slowly and after four to five weeks the rates of Cd adsorption by the sediment increased. High quantities of Cd in the water may have adsorbed onto particulate matter and competed with the sediment for cadmium. After two to three weeks the particulate matter may have become saturated with Cd and the Cd entering the system was then adsorbed mainly by the

sediment. Factors which lead to the increased rate of Cd adsorption by sediments are unknown other than the saturation effect that may have occurred in the water column. When metallic mercury was placed in a similar system there was a similar increased rate of adsorption of mercury by sediments at increased distances from the site of Hg addition (Titus et al., 1980), although when Hg(II) was added to the water as in this experiment, the adsorption rate was constant until the Hg concentration in the incoming water was increased. Mercury in sediments is known to be modified by reduction of Hg(II) to Hg^0 (Fang, 1973) and methylation of Hg(II) to CH_3Hg^+ (Jernelov, 1972). These forms of mercury are more mobile and may be passed more readily through the sediments than other forms of the metal. Whether a similar mechanism may occur with cadmium is not known, although a bacterium has been found to methylate cadmium (Heuy et al., 1974). Precipitation of Cd(II) as CdS may also have occurred in anaerobic portions of the sediments of the model aquatic system.

The numbers of organisms in the water column of the Cd-treated system increased while those in the control tank did not. If Cd caused lysis of some bacterial and phytoplankton species, these organisms could release growth factors which would lead to increased numbers of bacteria capable of growth in the water column in the presence of cadmium. The increased percentage of resistant bacteria in the Cd-treated system tends to support this hypothesis.

Sediment bacterial populations were not as affected by Cd as were the populations in the water column. The population remained constant (with some variation) until the cadmium level in the sediment was high. There was then an increase in the TVC/g as well as an increase in the percent

resistance of the sediment population. This is consistent with the increase in the water column, but the TVC and the percent of resistant bacteria decreased rapidly back to levels near those observed prior to the increase even though the Cd load of the sediment continued to increase. The sediment is apparently a much more protective environment than the water column. Experiments involved with adsorption of cadmium by bacteria and sediment showed that bacteria could adsorb more Cd on a dry weight basis than sediment, but the large ratio of sediment to bacteria in the model aquatic system would tend to adsorb cadmium so that high concentrations would not be available to the sediment bacterial populations. The percent resistant to Cd in the water column increased immediately after Cd exposure, but the sediment population did not respond until the total Cd concentration of the sediment reached over 100 µg Cd/g. This reaction was similar to that seen for sediment bacterial populations exposed to Hg (Titus et al., 1980).

The types of organisms detected that were resistant to 15 µg Cd(II)/ml on PCA were similar to those found in the Ottawa River sediments. The types of organisms isolated after ten weeks of Cd exposure were similar in both the control tanks and the treated tanks. More diverse populations were found in the sediment than in the water column and Bacillus sp. were prevalent in the sediment but not in the water. The genera present after ten weeks changed due to the aging process of the tanks, and this change seemed to affect the types of resistant organisms present more than the cadmium treatment.

Certain problems exist in doing plate counts and selecting isolates from plates for examination of a bacterial population, whether in nature

such as the Ottawa River sediments or in laboratory microcosm experiments. Serial dilution onto plates selected organisms which were present in the highest numbers, and in this study, which could form colonies on Plate Count Agar at 23°C. Therefore, the isolates obtained and identified may not reflect the true heterogeneity of the total bacterial population. Also, the limited number of isolates examined from the large number possible may not represent the total population completely.

The experiments involving adsorption of Cd from water by sediments and by a Cd-resistant bacterial isolate examined conditions which influenced the rate of Cd adsorption. The results suggested that those conditions under which Cd was most mobile induced the highest adsorption of Cd by both bacteria and sediments. The highest rates of adsorption were observed at a temperature of 35°C and at pH values of 6. At alkaline pH values Cd may form insoluble complexes with the carbonate present in the buffering system. These complexes may not have passed through the dialysis membrane to the bacteria and sediments as well as the free ionic form. The lower incubation temperature also lowered the amount of Cd adsorbed, probably due to the decreased rate of both biological and chemical processes at lower temperatures. Similarly, the increased rate of adsorption at higher temperatures was probably related to the increased rate of chemical and biological reactions at elevated temperatures.

The adsorption of Cd at lowered Eh values was examined, and it was found that these conditions accelerated Cd adsorption by bacteria and especially by sediment. One plausible explanation for this may be that phosphate and carbonate were replaced by sulfide as the controlling anionic species (within dialysis bags) under more reduced conditions (Lu

and Chen, 1977). A higher concentration of sulfide groups was available within the dialysis bags containing bacteria and/or sediments than in the phosphate-carbonate buffer. When Cd entered the sediment it precipitated as CdS and remained within the dialysis tubing. The level of Cd in the sediment would continue to increase as Cd continued to enter by diffusion without CdS being removed. This explanation assumes that an equilibrium is set up in the air-equilibrated treatments.

CONCLUSIONS AND SUMMARY

The results obtained in this study have shown that the effects of cadmium on natural sediment bacterial populationss and on laboratory microcosm populations can be related. Acclimation of bacteria to the toxic effects of cadmium may occur in both systems. Bacteria of diverse biotypes were found to be able to overcome the toxic effects of cadmium after exposure to the metal. Those bacteria which existed in the water column were affected by cadmium exposure more than sediment bacteria. Cadmium was adsorbed by bacteria and by sediment, but on a dry weight basis the bacteria adsorbed more cadmium than the sediment.

The significance of this work lies in a further understanding of how cadmium may influence bacteria in the environment (as well as in other systems such as waste treatment facilities) and of how bacteria may compete with particulate matter for available cadmium in the water column and interstitial water. The adsorption of cadmium by bacteria could result in the mobilization of cadmium into the food chain where the metal could accumulate in higher trophic levels and ultimately man.

APPENDIX A

Atomic Absorption Spectrophotometry

APPENDIX A

Atomic Absorption Spectrophotometry

A Perkin-Elmer (Norwalk, Conn.) Model 403 Atomic Absorption Spectrophotometer (AAS) was used to measure the level of the various metals in this study. Three forms of analysis were employed for various purposes in this report: direct aspiration, sample boat technique, and flame microsampling.

Direct Aspiration

Direct aspiration was used for analysis of digests of river sediment and for analysis of water from the model aquatic systems and the Cd uptake experiments in the reaction kettles. The sampling tube of the AAS was placed in the sample to be analyzed and the absorbance was noted from the digital readout. Linear regression analysis of five standards was used to ascertain the metal content.

Flame Microsampling

Flame microsampling was used to analyze Cd in model aquatic system sediments, artificial sediments, and bacteria digested with HNO_3. The system was based on that described by Voth (1981) for the analysis of samples containing high levels of dissolved solids. A 50 µl acid digested sample was placed in a 1.5 ml polypropylene micro test tube, 50 µl of water or water-Cd were added, and the 100 µl diluted sample was completely aspirated into the flame. Peak heights were recorded and Cd analyzed as for the boat technique.

Method of Standard Additions

Standard additions were used to alleviate interferences which could lower the amount of Cd found in samples. This is generally due to the

inability to make the standard matrix similar enough to the samples matrix. The method involves adding a known quantity of Cd to the sample and aspirating. In flame microsampling the standard additions used were 0, 30, and 60 ng Cd (in 50 ul ddH_2O), and in the boat technique additions of 0, 2, and 4 ng Cd were used. At least 3 separate analyses were performed for each addition, and adsorbance vs standard addition were used in linear regression analysis to find the Cd level using the equation for the slope of the line described by the regression analysis. By substituting into the equation and solving for ug Cd when adsorbance was 0, the amount of Cd in the sample was ascertained (using the absolute value of the negative integer obtained).

TABLE 7

STANDARD OPERATING CONDITIONS FOR ATOMIC ADSORPTION SPECTROPHOTOMETRY

Element	Lamp*		Wavelength (nm)	Slit	Current (mA)
	Manufacturer	Part No.			
Cd	Westinghouse	WL-36016	228.8	4	10
Fe	Perkin-Elmer	303-6037	248.3	3	30
Na	Perkin-Elmer	303-6[illegible]	589.3	4	10
Pb	Westinghouse	WL-36039	283.3	4	8
Zn	Perkin-Elmer	303-6093	213.9	4	15
Cr			357.9	4	
Cu	Perkin-Elmer	303-6096	324.8	4	30
Ni			232.0	3	

* All lamps were Hollow Cathode lamps.

Flame consistency Air-acetylene	Cr	Reducing	Air --- 23 l/min Acetylene --- 8.8 l/min
	Others	Oxidizing	Air --- 23 l/min Acetylene --- 7.2 l/min

APPENDIX B

Supplemental Data from Preliminary

Sampling Trips.

TABLE 8

PRELIMINARY DATA

Date Collected	Sample ID	Distance from Outflow	µg Metal per gram of sediment							% OM	CEC mEq 100g
			Cd	Cr	Cu	Ni	Pb	Zn	Fe		
07/12/79	01-1A	10 m	4.80	742.5		590.0					
	01-1B	20 m	6.12	985.0		592.5					
	01-2	1.61 km	1.88	101.3		84.8					
	01-3	4.8 km	1.28	31.8		73.1					
	01-4	6.8 km	2.42	205.0		93.5					
10/07/79	02-1	20 m	5.55	434.1	48.2	348.3		118.0	18400		
10/29/79	03-01	-12 m	3.71	14.1	6.9	56.3		35.6	10332	1.9	8.7
	03-02	-90 m	2.76	23.0	33.2	106.5		119.4	9123	2.2	3.5
	03-1	20 m	4.88	113.8	79.0	370.6		378.5	16542	8.5	13.0
	03-2	0.16 km	3.00	42.3	18.4	72.1		533.5	15680	1.6	9.1
	03-3	0.32 km	3.80	1307.3	21.3	-----		307.8	-----	5.0	7.8
	03-4	0.48 km	3.45	66.9	27.5	-----		507.8	-----	4.3	11.3
	03-5	0.64 km	1.25	69.8	21.9	-----		130.8	-----	2.4	8.3
	03-6	0.80 km	3.99	25.7	23.2	55.7		104.0	15063	3.7	11.7
	03-7	0.96 km	3.99	66.6	30.9	69.2		223.5	14678	3.2	7.0
	03-8	1.12 km	6.81	35.7	33.3	85.0		130.5	11849	2.4	10.4
	03-10	1.44 km	0.12	17.7	7.9	-----		60.0	-----	3.9	10.4
	03-11	1.61 km	2.44	22.4	8.8	55.1		50.2	10563	1.4	11.3
12/08/80	04-1	12 m	3.01	168.0	55.3	217.4	232.0	307.6	30500	7.6	
	04-2	0.16 km	1.88	15.4	8.8	31.7	29.2	203.5	23000	1.9	
	04-3	0.32 km	4.46	302.9	45.0	85.3	160.5	605.7	38600	5.5	
	04-4	0.48 km	4.82	17.6	183.1	48.6	247.5	268.7	34200	15.7	
	04-5	0.64 km	2.21	19.2	20.8	44.6	94.6	205.7	21300	3.2	
	04-6	0.80 km	3.39	22.7	23.8	34.6	289.6	140.6	33600	3.8	
	04-7	0.96 km	2.34	35.6	14.6	42.4	133.1	97.0	25800	2.4	

Table 8 - Continued

Date Collected	Sample ID	Distance from Outflow	µg Metal per gram of sediment							% OM
			Cd	Cr	Cu	Ni	Pb	Zn	Fe	
12/08/80	04-8	1.12 km	3.00	88.3	23.6	78.9	95.4	239.2	27800	2.0
Continued	04-10	1.44 km	2.62	100.5	31.8	64.7	208.4	270.4	23800	4.0
	04-11	1.61 km	2.77	242.5	30.8	16.2	191.1	213.9	24000	5.6
04/05/80	05-1	1.5 m	2.91							
	05-2	1.0 m	1.66							
	05-3	7.0 m	7.32							
05/24/80	06-1	11.3 km	2.37							
	06-2	11.2 km	1.62							
	06-3	9.7 km	3.25							
	06-4	9.6 km	3.68							
	06-5	6.8 km	3.66							
05/30/80	07-1	11.2 km	0.76							
	07-2	11.2 km	0.94							
	07-3	11.3 km	0.64							
	07-4	11.3 km	0.77							
	07-5	12 km	2.87							
	07-6	12 km	2.93							
	07-7	12 km	3.40							
06/21/80	08-1	11.3 km	4.04	10.6	52.5	21.0	480.4		21800	
	08-2	11.3 km	3.93	11.6	34.3	20.9	355.2		18500	
	08-3	11.3 km	3.72	14.3	38.0	23.5	330.8		19200	
	08-4	11.3 km	4.85	10.7	32.2	23.0	181.9		20700	
	08-5	7.0 m	5.76	746.3	60.7	159.0	255.4		22200	
	08-6	7.9 m	8.92	1120.1	68.0	137.7	288.1		21900	

APPENDIX C

Supplemental Data from Sampling Trips

TABLE 9

SUPPLEMENTAL DATA

Date Collected	Sample Identification	pH	Temperature (°C)	Eh (mV)
07/01/80	09-1	7.10	27.0	-297
	09-2	7.30	30.1	-367
08/01/80	10-1	7.17	25.9	-376
	10-2	7.10	30.6	-320
09/05/80	11-1	7.18	26.3	-254
	11-2	7.34	26.7	-303
10/01/80	12-1	6.60	25.3	-346
	12-2	7.05	26.5	-400
11/07/80	13-1	6.97	11.5	-196
	13-2	7.61	21.0	-270
12/02/80	14-1	6.95	10.3	-111
	14-2	7.25	17.2	-233
01/02/81	15-1	6.32	4.5	-137
	15-2	7.09	16.3	-210
02/02/81	16-1	6.65	3.8	-122
	16-2	7.11	15.8	-205
03/11/81	17-1	6.71	12.6	-165
	17-2	7.15	18.7	-225
04/08/81	18-1	7.11	14.5	-297
	18-2	7.21	24.4	-262
05/11/81	19-1	6.87	13.9	-278
	19-2	7.00	18.5	-304
06/20/81	20-1	6.87	27.4	-226
	20-2	7.27	28.2	-274

APPENDIX D

Adsorption of Cadmium by Bacteria and Sediments Under Various Conditions

TABLE 10

Cd ADSORPTION AT pH 7 AND 23°C

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/g	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	7.00			1.06	1060					1060.0	100.0
1	7.01			1.05	1050					1050.0	99.1
2	7.04			1.04	1040					1040.0	98.1
4	7.05	297	22.8	1.05	1050					1050.0	99.1
0	6.98			1.04	1040					1040.0	100.0
1	7.03			1.03	1030	0.24	10.4			1040.4	100.0
2	7.03			1.01	1010	0.46	16.8			1026.8	98.7
4	7.05	267	22.8	1.00	1000	1.18	27.3			1027.3	98.8
0	7.10			1.04	1040			3.35		1040.0	100.0
1	7.03			0.99	990			47.97	40.2	1030.2	99.1
2	7.01			0.93	930			95.50	60.0	990.0	95.2
4	7.02	298	24.2	0.91	910			245.94	109.0	1019.0	98.0
0	7.01			1.01	1010			3.46		1010.0	100.0
1	7.05			0.94	940	0.33	14.2	43.68	36.2	990.4	98.1
2	7.03			0.90	900	0.54	20.4	97.51	68.5	988.9	97.9
4	7.06	273	24.2	0.87	870	1.21	30.2	176.53	92.2	992.4	98.3

TABLE 11

Cd ADSORPTION AT pH 6 AND 23°C

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/g	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	6.01			0.99	990					990.0	100.0
1	6.03			0.98	980					980.0	99.0
2	6.03			0.98	980					980.0	99.0
4	5.97	378	22.6	0.97	970					970.0	98.0
0	5.98			0.98	980					980.0	100.0
1	6.00			0.92	920	1.04	46.8			966.8	98.7
2	6.00			0.91	910	1.58	63.0			973.0	99.1
4	5.99	316	22.9	0.90	900	2.12	71.1			971.1	99.1
0	5.99			1.00	000			2.95		1000.0	100.0
1	5.99			0.93	930			66.38	57.1	987.1	98.7
2	5.99			0.90	900			117.90	88.0	988.0	98.8
4	5.98	366	24.5	0.86	860			210.33	115.7	975.7	97.6
0	5.98			1.00	000			3.70		1000.0	100.0
1	6.00			0.86	860	1.22	76.6	64.10	54.8	991.3	99.1
2	6.01			0.83	830	1.70	62.1	103.73	78.6	970.7	97.1
4	5.98	324	24.6	0.79	790	2.45	80.6	206.20	109.3	979.9	98.0

TABLE 12

Cd ADSORPTION AT pH 8.5 and 23°C

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/g	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	8.45			1.12	1120					1120.0	100.0
1	8.49			1.08	1080					1080.0	96.4
2	8.48			1.04	1040					1040.0	92.9
4	8.58	302	22.6	1.00	1000					1000.0	89.3
0	8.47			1.12	1120					1120.0	100.0
1	8.51			1.10	1100	0.09	3.8			1103.8	98.6
2	8.50			1.10	1100	0.16	5.8			1105.8	98.7
4	8.59	310	23.0	1.01	1010	0.24	7.0			1017.0	90.8
0	8.50			1.01	1010			4.00		1010.0	100.0
1	8.53			0.99	990			9.60	5.0	995.0	98.5
2	8.53			0.98	980			11.27	6.0	986.0	97.6
4	8.52	316	24.1	0.96	960			20.13	8.7	968.7	95.9
0	8.50			1.07	1070			3.33		1070.0	100.0
1	8.52			1.04	1040	0.16	6.8	8.23	4.4	1051.2	98.2
2	8.51			0.98	980	0.14	6.2	11.53	6.4	992.6	92.8
4	8.59	307	24.2	0.97	970	0.31	8.7	24.93	10.4	989.1	92.4

TABLE 13

Cd ADSORPTION AT pH 7 AND 4°C

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/b	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	6.98			1.04	1040					1040.0	100.0
1				0.98	980					980.0	94.2
2				0.98	980					980.0	94.2
4	5.97	318	4.2	1.00	1000					1000.0	96.2
0	6.96			1.02	1020					1020.0	100.0
1				1.01	1010	0.31	10.0			1020.0	100.0
2				0.97	970	0.39	11.7			981.7	96.2
4	5.80	300	4.3	0.94	940	0.51	12.9			952.9	93.4
0	6.96			1.02	1020			1.37		1020.0	100.0
1				0.98	980			37.93	32.9	1012.9	99.3
2				0.95	950			56.60	41.1	991.1	97.2
4	5.69	305	4.2	0.94	940			96.70	56.1	996.1	97.7
0	6.92			1.02	1020			1.73		1020.0	100.0
1				0.97	970	0.31	10.0	42.23	36.5	1016.5	99.7
2				0.94	940	0.34	10.6	58.07	45.9	986.5	96.7
4	5.5	308	4.3	0.89	890	0.49	12.2	94.60	56.9	959.1	94.0

TABLE 14

Cd ADSORPTION AT pH 7 AND 35°C

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/g	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	7.05			1.08	1080					1080.0	100.0
1				1.07	1070					1070.0	99.1
2				1.01	1010					1010.0	93.5
4	7.26	291	34.1	1.00	1000					1000.0	92.6
0	7.05			1.00	1000					1000.0	100.0
1				0.96	960	0.71	22.8			982.8	98.3
2				0.94	940	1.17	32.6			972.6	97.3
4	7.26	289	33.8	0.90	900	2.73	49.3			949.3	94.9
0	7.07			1.02	1020			1.73		1020.0	100.0
1				0.83	830			174.43	151.2	985.4	96.6
2				0.80	800			266.73	210.8	1010.8	99.1
4	7.25	292	34.4	0.72	720			411.63	254.3	974.3	95.5
0	7.06			1.03	1030			1.97		1030.0	100.0
1				0.84	840	0.61	19.6	151.17	134.3	993.9	96.5
2				0.77	770	0.92	26.2	242.63	189.2	985.4	95.7
4	7.25	292	33.9	0.68	680	2.28	40.8	444.90	249.8	970.6	94.2

TABLE 15

Cd ADSORPTION AT pH 7, 23°C, AND LOW Eh

Day	pH	Eh (mV)	Temp. (°C)	Water µg Cd/ml	Water Total µg Cd	Bacteria µg Cd/mg	Bacteria Total µg Cd	Sediment µg Cd/g	Sediment Total µg Cd	Total µg Cd Recovered	Percent Recovered
0	7.02	30		0.99	990					990.0	100.0
2	7.00	96	23.1	0.98	980					980.0	99.0
0	7.04	30		0.98	980					980.0	100.0
2	7.01	90	23.2	0.95	950	0.85	22.3			972.3	99.2
0	7.03	30		0.99	990			0.77		990.0	100.0
2	7.03	92	24.2	0.66	660			348.37	311.2	971.2	98.1
0	7.03	30		0.99	990			1.07		990.0	100.0
2	7.01	95	24.1	0.67	670	1.04	27.3	280.90	249.6	946.9	95.6

APPENDIX E

Review of Literature Concerning Cadmium In Plants and Aquatic Biota

APPENDIX E

LITERATURE REVIEW

Properties of Cadmium

Cadmium is a member of Group II-B in the Periodic Table along with zinc and mercury. Ionic cadmium exists only as the 2+ valence state. Selected properties of Cd are outlined in Table 16. Concentrations of Cd less than 10 mg/l may be stable in water that has a low solute concentration and/or pH. This may account for the difficulty in removing low levels of Cd from water by conventional water treatment processes (Hem, 1972). Gardiner (1974a) has shown that a substantial proportion of the total Cd found in river and lake waters is present as the free Cd(II) ion. The concentration of free Cd(II) increases as the pH of the water is lowered and decreases with increasing solutes. It has been shown in Precambrian Shield lakes in Ontario, Canada, that natural Cd levels of 1.4 ppb exist and that about 50% is strongly bound and 50% is liable (Chau and Lum-Shue-Chan, 1974). Humic substances account for most Cd complexation in natural waters, followed by carbonates (Gardiner, 1974a).

Sources of Metals in the Environment

Natural Sources

Natural metal enrichment in the environment may arise from the following sources (Goldberg, 1954): (a) lithogenous formations - weathering products from source areas or rock debris from river beds; (b) hydrogenous formations - particulate matter, precipitation products, and/or adsorbed substances formed due to physio-chemical changes in waters; (c) biogenous formations - remains of biological organisms, decomposition products or organic substances, and inorganic siliceous or

TABLE 16

PROPERTIES OF CADMIUM

Atomic number	48
Atomic weight	112.40
Density (g/cc)	8.65
Boiling point (^{o}C)	765
Melting point (^{o}C)	320.9
Oxidation states	0, +2
Atomic radius (Å)	1.54
Ionic radius (Å)	0.97
First ionization energy (kcal/g-mole)	207
Heat of vaporation (kcal/g-atom)	23.9
Heat of fusion (kcal/g-atom)	1.46
Crystal structure	hexgonal
Electronegativity (Pauling's)	1.7
Vapor pressure @ 400^{o}C (mm of Hg)	1.4
Natural occurring isotopes isotope (abundance)	106 (1.22%), 108 (0.88%), 110 (12.39%), 111 (12.75%), 112 (24.07%), 113 (12.26%), 114 (28.86%), 116 (7.58%)
Radioactive isotopes	109, 115m
Common associated anions	Cl^{-}, S^{-2}, NO_3^{-}, O^{-2}, OH^{-}, CO_3^{-2}

calcareous shells; (d) atmogenous formations - resulting from atmospheric fallout; and (e) cosmogenous formations - extraterrestrial particles. Man-made effects may influence metal enrichment in the first four categories listed (Forstner and Whittman, 1979).

Atmospheric deposition of cadmium is thought to be the major source of Cd in the environment (Fleischer et al., 1974). World wide annual emission of Cd has been estimated at 80×10^5 kg, 90% of which is from anthropogenic sources (Nriagu, 1980). Ranges of Cd in air have been reported up to 600 ng/m in highly industrialized areas (Friberg et al., 1971) although levels of 23-370 ng/m^3 in these urban areas are more common (Harrison, 1973). The cadmium levels in the air of rural areas is lower than urban, averaging about 4 ng/m^3 (Harrison, 1973). Natural sources include vegetation, airborne soil particulates, forest fires, and volcanic aerosols (Nriagu, 1980). Anthropogenic sources of Cd in the atmosphere are mainly due to smelting operations (Fleischer et al., 1974; Lagerwerff and Brower, 1974), industrial burning of fossil fuels (Fleischer et al., 1974), and automotive exhausts (Peyton et al., 1974). Aerial input of cadmium into a burrow pit near Gary, Indiana, has been shown to be between 0.08-0.27 mg per m^2 per month. Over a 19 year period this rate lead to the aerial deposition of 1.92 kg Cd, a value which accounted for 69% of the total Cd found in the sediments of the pit (Peyton et al., 1974).

The other major anthropogenic source of cadmium is direct contamination of aquatic systems. Effluents from primary sewage facilities and industries are known to pollute aquatic systems (Jones et al., 1973; Kneip and Hernandez, 1974; Nelmes, 1974). Runoff from mines (Babich and Stotzky, 1978), from phosphate fertilized agricultural lands

(Friberg et al., 1971; Hutchinson et al., 1974), from fields having sewage applied as a fertilizer (Jones et al., 1973; Metcalf, 1974), and from urban areas (Chen et al., 1974) contribute to Cd enrichment. Cadmium may enter the environment from a variety of commercial sources including industries involved with electroplating, nickel-cadmium batteries, ceramics, pigments, production of alloys of Cu, Pg, Ag, Al, and Ni (Harrison, 1973), brewing, tanning, and chemical production (Fitchko and Hutchinson, 1975).

Levels of Cd in Waters and Sediments

Investigators have examined the levels of cadmium in various locations throughout the world. Fleischer et al. (1974) and Harrison (1973) reported Cd levels of 0.2 ppm for the earth's crust, 0.3 ppm for soil, 1 ug/l (ppb) in fresh water, an average of 0.15 ppb in sea water, and about 2 ppm in coal. Tables 17 and 18 show selected levels of Cd in both nonpolluted and polluted waters and sediments of various locales throughout the world.

Various physical-chemical and anthropogenic factors affect the levels of Cd and other metals in aquatic systems. In ocean water the cadmium concentration is highly correlated with phosphate and nitrate concentrations in the water (Bruland et al., 1978). Kemp et al. (1976) have shown a positive correlationship between the cadmium level and organic carbon in Lake Erie sediment. Vivian and Massie (1977) have studied the River Tawa in South Wales which has several sources of metals and found (a) increases in nickel and copper due to a nickel refinery, (b) increases in Cd, Zn, Cu, and Pb due to a tributary containing a high concentration of dissolved trace metals, and (c) increases in Cd, Cu, and

TABLE 17

LEVELS OF CADMIUM REPORTED IN SEDIMENTS

Place	Type	Range (ppm)	Ave. (ppm)	Reference
Shoreline sediments of San Francisco Bay	top bottom	0.06-4.69	1.22	202
Lake Indiana sediments	contaminated noncontaminated		969 4	284
Shale			0.74	280
Shale			0.22	181
North Atlantic			0.225	5
Rhein River sediments	nonpolluted	0.21-0.63	0.37	76
Lake Germany sediments			0.24	204
Plein River	polluted (due to pigment prod. plant)		300	77
Great Lakes: Huron			0.5	37
			2.4	137
St. Clair			1.7	263
Michigan			2.3	219
Erie			4	138
Cleveland Harbor			11 (Max)	282
Ontario			5.2	137
Foundry Cove, New York	NiCd battery plant outflow	3,000-50,000	25,700	148
Sorfiord, Norway			850	251

TABLE 18

LEVELS OF CADMIUM REPORTED IN WATERS

Place	Type	Range (mg/l)	Ave. (mg/l)	Reference
Remote California Streams		0.01-0.03		139
Mississippi River			0.1	267
Amazon River			0.07	29
Lake - Japan			0.02	259
Lake - Canada			0.1	18
Lake - U.S.A.			0.02	242
Foundry Cove, New York	Ni-Cd battery plant outflow			
	Ebb		11.9 (filterable)	
			55.8 (particulate)	
	Flood		5.6 (filterable)	103
			9.6 (particulate)	

Zn due to effluent from a steel mill. Levels of Cd, Zn, Pb, and Cu are lower in pre-cultural lakes than in post-cultural lakes of Wisconsin, an effect due primarily to industry in the southern portion of the state (Iskander and Keeney, 1974). The levels of various metals in sediments of the ocean may be used to follow contamination by waste materials. Mercury has been used to follow the rate of sewage sludge addition and to trace the sources of pollution in the ocean (Klein and Goldberg, 1970). Gross et al. (1972) have shown that Pb in the New York Bight is the best indicator of waste material contamination. Enrichment of 1-10 ppm of Cd in ocean sediments is commonly due to the proximity of domestic and industrial sewage outflows (Forstner, 1980).

Levels of cadmium in the water systems of Ohio have also been studied. Kemp and Thomas (1976) and Kemp et al. (1976) reported levels from 1.6 to 6.2 ppm in the upper portion of Lake Erie sediments. Wilson and Walters (1978) examined water and upper sediments of Lake Erie and found about 0.1 ppb and 8-9 ppm, respectively. The background levels of Cd in the Maumee River basin of northwestern Ohio have been found to be 0.011 ppm in water and 0.098 ppm in bottom sediments (Naymik, 1977).

Studies of various types of metal contaminations have shown there is a correlation between Cd pollution and other metals. Forstner and Muller (1973) have used an "index of relative pollution potential," i.e., the ratio of metal consumption to average metal content in a specific sphere, to show that Cd, Hg, Pb, Zn, and Cu are the most prevelant metal contaminants in the environment. The order of metal enrichment of sediments by anthropogenic sources has been given as Cd > Pb > Zn > Cu, which corresponds to accumulations of metal in fossil fuels (Suess and

Erlenkeuser, 1975). Mercury enrichment by man is probably greater than Cd enrichment (Forstner, 1980).

Cadmium in Waste Water and Sewage Sludge

Domestic and industrial effluents are known to contain varying levels of Cd and other heavy metals. Metal input to sewage treatment plants is not a continuous process, but generally consists of slugs of metal (Oliver and Cosgrove, 1974). A conventional activated sludge plant removes metals in: (a) primary settling which removes insoluble metal or metal adsorbed to particulates (Oliver and Cosgrove, 1974) and (b) secondary settling following aeration which removes metals adsorbed to the biological floc formed during aeration (Chen et al., 1974). Lead, copper, chromium, mercury, cadmium, and arsenic are reduced to low levels by precipitation with either $Al_2(SO_4)_3$ or $Ca(OH)_2$ (Nilsson, 1971). Domestic effluents which also included light industries have been shown to increase the cadmium level in river sediments to 5-10 ppm (Forstner, 1980). The application of fertilizer and sewage sludge for seven years to a salt marsh in New York resulted in the retention of 20-35% of the added Cd, 20-50% of Cr, 60-100% of Cu, 55-100% of Pb, 80-100% of Fe, 55-60% of Ni, and 20-45% of Zn (Giblin et al., 1980).

Cadmium in Soils

Cadmium levels in soils vary greatly with local contamination. The proportion of the Cd which is available for uptake by plants is of great interest. When cadmium was adsorbed to the humic fraction of soils, approximately 50% was exchangeable and about 50% was in coordination complexes (Riffaldi and Levi-Minzi, 1975). Cadmium was exchanged better when Ca(II) was present in the soil solution than when Al(III) was

present, while as the Na concentration decreased, Cd(II) adsorption also decreased (Lagerwerff and Brower, 1972). Positive correlations of the amount of Cd adsorbed by ten Canadian soils were found for cation exchange capacity, pH, and organic carbon (Singh, 1979). Hutchinson et al. (1974) showed that Pb, Cd, Zn, Cu, and Ni are concentrated in various vegetables when grown in metal contaminated soils.

Transport, Distribution, and Cycling of Cadmium

The distribution of cadmium from point sources may be affected mainly by physical parameters. Tidal flows have been shown to be a significant factor in distributing Cd from a cove to the open ocean (Kneip et al., 1974). In the Corpus Christi Bay, which had high Zn and Cd levels, there was a large concentration gradient in the bay, with the highest levels near the harbor entrance. In the summer, stagnation of the harbor water increased the metal concentration because a significant proportion precipitated due to reducing conditions. Winter conditions lead to an exchange of water between the bay and harbor, and metals desorbed from harbor deposits were washed into the bay where they were readsorbed by particles settling to the bottom (Holmes et al., 1974).

Cycling of cadmium in microcosms has been studied by Huckabee and Blaylock (1973, 1974). These authors added Cd as simulated rainfall to the terrestrial portion of a terrestrial-aquatic microcosm. The terrestrial portion retained 94-96% of the added cadmium, with the order of Cd concentration as soil > moss > litter > higher plants. In the aquatic portion of the microcosm, the order of Cd concentration was sediment > water > snails > watercress ≥ fish.

Transfer of Cd to animals may be related to the uptake of

contaminated food sources. Levels of Cd in animal rations reported by Sharma (1980) were (in μg/g): hay, 0.18; dairy grain ration, 0.17; soybean meal, 0.19; commercial hog ration, 0.23; commercial chicken ration, 0.32. Gish and Christensen (1973) have shown that the 11-fold increased concentration of Cd in earthworms from soil was significantly correlated with soil Cd concentration and soil organic matter. The consumption of contaminated earthworms by predators such as birds, amphibians, reptiles, or mammals, may represent a possible mechanism for the translocation of cadmium from the soil to higher animals.

Levels of cadmium in various trophic levels of aquatic ecosystems have been investigated. Enk annd Mathis (1977) studied Jubilee Creek in Illinois and found that aquatic insects contained an average of 0.98 ppm Cd; fish, 0.16 ppm; sediment, 0.14 ppm; and water, 0.02 ppm. Guthrie et al. (1979) used a marine microcosm to study cadmium distribution through various types of organisms. Levels reported were (in mg Cd/kg wet weight): water, 1.17; sediment, 1.88; barnacle, 1.19; crab, 0.14; oyster, 0.48; clam, 1.19; polychaete, 1.75. A model for cadmium dispersal through a Lake Erie food chain has been reported by Thomann et al. (1974). The percentages of the total mass for various systems were: water, 37%; phytoplankton, 24%; zooplankton, 13%; fish, 15%; lake birds, 1%; and bottom sediments, 10%. A scheme for the passage of cadmium through a system in which the main source of Cd was from a Zn-Pb smelting operation included compartments for water, air, soil-sediment, fish, animals, and man (Moyer and Budinger, 1974).

Factors Affecting Mobility of Metals

Residence Times in Aqueous Phase

The amount of time that cadmium or other metal remains in waters is given by the formula

$$T = A / dA/dt$$

where T is the residence time, A is the total amount of element in suspension or solution in a body of water and dA/dt is the amount of element introduced within a specific interval of time (Forstner and Whittman, 1979). Residence time calculations should include the following variables (Bowen, 1977): (a) surface and ground water flows; (b) atmospheric fallout and evaporation; and (c) sedimentation and remobilization. Using calculations such as these along with other experimental data, Forstner and Whittman (1979) stated that Al, Ti, Cr, and Fe have relatively short residence times, while Cd, Li, U, Sb, and Na have relatively long residence times.

Types of Associations in the Environment

1. Heavy metals in detrital minerals. Heavy metals may become incorporated into the lattice of clay minerals where they are relatively inert. Chromium and copper bind strongly to illite, while V, Co, and Ni bind to montmorillonite (Hirst, 1962).

2. Precipitation of heavy metals. The precipitation of cadmium and other heavy metals is dependent on pH and on anionic species present (Forstner and Whittman, 1979). Anionic species which are prevalent in surface waters and pore solutions are chlorides, sulfates, carbonates, and, under reducing conditions, anionic species from H_2S (Forstner and Whittman, 1979). Hydroxides form insoluble precipitates with metals at pH 9-12 and completely dissolve at about pH 4 (Jenne, 1968). Metal sulfides are practically insoluble at neutral pH. Cadmium sulfide is soluble in

HCl, while Cu, Pb, and Hg are only soluble in oxidizing acids such as HNO_3 (Jenne, 1968). In fresh waters the controlling anionic species is the carbonate ion (Weber and Possett, 1974) and the generalized equilibrium is given as

$$Me\ (II)\ CO_3 + H_2O + CO_2 \rightleftarrows Me(II)\ (aq) + 2\ (HCO_3^-)\ (aq)$$

All heavy metal carbonates are more soluble in the presence of CO_2 (Forstner and Whittman, 1979). Popava (1961) showed that coprecipitation with $CaCO_3$ can adsorb as much as 100% of the Cd in water.

3. Cation exchange and adsorption. Adsorption is the ability of fine-grained materials with large surface area to accumulate heavy metal ions at the interface between solid and liquids due to intermolecular forces (Forstner and Whittman, 1979). The exchange capacity of a substance is its sum of exchangeable cations or anions. Substances in waters capable of exchanging cations include (Forstner and Whittman, 1979): (a) clay minerals, (b) freshly precipitated Fe-hydroxides, and (c) organic substances. Scheffer and Schachtschabel (1966) outlined factors which affect the affinity of cations towards exchanges: (a) valence and hydration effects - valence controlled affinity $Me^+ < Me^{2+} < Me^{3+}\cdots$ and hydration (decreases in diameter) Ba < Sr < Ca < Mg < Cs < Rb < K < Na < Li; (b) concentration in solution - as the concentration of a metal in solution increases, the numbers of exchanged cations also increases; (c) reactions involving hydrolized cations - hydroxyl complexes of heavy metals may be more readily sorbed than free ions; (d) specific reactions between inorganic exchanges and cations - heavy metals generally are more readily sorbed than the metals of the alkali and alkaline earth groups.

4. Sorption onto clay minerals. The capacity of clay minerals to

sorb cadmium and other metals is governed (a) by broken bonds around edges of silicon-aluminum units and (b) by substitution of Si^{4+} by Al^{3+} in the tetrahedral layer (Grim, 1968). In both of these instances divalent cations balance the free charges in the minerals by sorption. Mitchell (1964) reported that the affinity of certain metals for clay minerals was Pb > Ni > Cu > Zn. It has been suggested that significant sorption of heavy metals onto clay minerals within water does not occur, probably due to other processes which reduce the concentration of metal in water prior to sorption by clay minerals (Block and Schnieder, 1967).

5. Sorption and coprecipitation on hydrous Fe/Mn oxides and Fe sulfides. These compounds can act as sinks for heavy metals in aquatic systems. They can readily sorb or coprecipitate both cations and anions and, when present even in low percentages as $Fe(OH)_2$ or MnO_2, may have the controlling influence on distribution of metals in an aquatic system (Lee, 1974). Under reducing conditions, hydrous Fe/Mn oxides are major sources of dissolved metals, particularly when high concentrations of dissolved organic matter is present (Jenne, 1976).

6. Metal associations with organic substances. Forstner and Whittman (1979) outlined five effects of dissolved organic substances on heavy metals: (a) complexation of metals, leading to increased metal solubility; (b) alteration of the distribution between oxidized and reduced forms of metals; (c) alleviation of metal toxicity altering metal availability to aquatic life; (d) influence on the quantity of metal adsorbed to suspended matter; and (e) influence on the stability of metal-carbon colloids. The order of stability of metal-organic matter complexes has been reported to be Pb > Cu > Ni > Co > Zn > Fe > Mn > Mg

(Irving and Williams, 1948). Goldberg (1965) has shown that marine organisms concentrate metals according to the above succession. Three processes which lead to the incorporation of a metal-organic species into sediments have been listed by Saxby (1973): (a) reactions between metal ions and organic ligands which can precipitate together directly or adsorb onto sediment; (b) incorporation of all or part of biological organisms containing metal coordination compounds into the sediments; (c) solubilization of metal-containing minerals by natural waters containing organic ligands, leading to adsorption of the metal by sediment molecules.

Chemical Factors Affecting Mobilization From Sediments

The mobilization of cadmium and other heavy metals from sediments into waters is of importance because these metals are generally hazardous to aquatic and terrestrial life. Four factors leading to the remobilization of heavy metals from sediments to waters have been outlined by Forstner and Whittman (1979).

1. Saltwater-sediment interactions. Two processes are effective in the release of heavy metals from sediments and waste particulates when in the presence of sea water (Rohatgi and Chen, 1975): (a) oxidation of either organic particulates with adsorbed metals or metal-sulfides and surface desorption of metals caused by a high dilution ratio and (b) complexation of heavy metals to form soluble complexes of inorganic ligands such as Cl^- and organic chelators. Changes in the concentration of different cadmium species at fresh water - sea water interfaces also leads to mobilization of the metal. Causes for these changes include different ionic strengths (Khalid, 1980), a lower concentration of suspended matter in the estaurine area (Windom, 1975), and different

concentrations of various major cations and ligands. The third factor is probably the factor which most controls the species of cadmium present in the marine environment (Khalid, 1980).

2. Metal release due to changes in oxidation-reduction (redox) potential. The redox potential of a body of water is influenced and controlled by biological utilization of oxygen, mixing of water due to wind and wave action, and depth of the water. As oxygen is consumed, the redox value of water and sediments decreases. Under oxidizing conditions the species of Cd is controlled by the concentration of various anions (Hem, 1972; Lu and Chen, 1977; Weber and Possett, 1974); however, under reducing conditions the controlling anionic species for Cd, as well as Hg and Pb, is sulfide (Lu and Chen, 1977). Using laboratory systems, Khalid et al. (1978) showed that as the oxygen content of a gas mixture passing over sediment material increased, there was a decrease in the pH value and were increases in both Cd content of the overlying water (25-30%) and redox potential.

3. Release of metals by acidic waters. The pH of an environment may become more acidic by at least two sources, acid mine drainage and acidic precipitation (acid rain). This generally leads to the increased solubility of cadmium and other heavy metals (Forstner and Whittman, 1979). Acidic conditions in the environment lead to problems related to increased availability and toxicity of cadmium with respect to plants (Linmann et al., 1973; Lucas and Davis, 1961). Increased acidity in aquatic systems may also accelerate the methylation of mercury by the bacterial population in sediments (Fagerstrom and Jernelov, 1972).

4. Mobilization by organic complexing agents. Organics in waters

may lead to solubility of metals by reduction of the element to a lower valence state as in the reduction of Fe and Mn by tannic acid (Hem, 1960; Rawson, 1963) or vanadium by peat, lignite, and humic acids (Szalay and Szilagyi, 1967). Organics may also cause solubilization by forming complexes with metal-containing suspended particles (Rashid and Leonard, 1973). The complexes formed by metals and humic acids are more stable than and may replace metal-inorganic ligands (Reuter and Perdue, 1977). Nitrilotriacetic acid (NTA) has been used as an alternative to polyphosphate in detergents and has been shown to mobilize Cu, Cd, Ni, Zn, and Pb from sediments, but not Cr (Banat et al., 1974). NTA also has been shown to increase the toxicity of Hg to the photosynthetic capacity of algae (Hongve et al., 1980).

Mobilization by Microbial Activity

Bacteria have been implicated in the mobilization of metals from sediments to the water column. Three microbial activities may influence the mobilization. Bacteria may cause breakdown of detrital organic matter to lower molecular weight compounds such as humic acids. Humic acid is more capable of complexing cadmium than are its less recalcitrant precusors (Riffaldi and Levi-Minzi, 1975).

Bacteria may influence their environment by changing the pH or redox potential. The oxidation of ferrous sulfide (FeS) by Thiobacillus ferrooxidans has been shown to produce acid which in turn may cause the breakdown of CdS, ZnS, and PbS (Malouf and Prater, 1961). The production of acid from pyrite by thiobacilli is the rate determining step for the microbial extraction of uranium from ore (Fisher, 1966). Thiobacillus thiooxidans can leach CdS in the presence of sulfer, with the amount of Cd

extracted increasing with a lowering of the pH or with a large initial inoculum (Brissette et al., 1971). The effects of lowering the redox potential have already been discussed (p.). McLerran and Holmes (1974) have reported that marine bacteria were capable of precipitating Cd and Zn, probably as CdS and ZnS.

The direct action of bacteria on various metals may result in the conversion of inorganic forms of the metal. An obvious example is the reduction of mercuric ion to elemental mercury (Kimura and Miller, 1964), the conversion of organomercurics to Hg(II) and then to Hg (Furukawa et al., 1969; Matsumra et al., 1971; Spangler et al., 1973), and the methylation of Hg(II) to form methyl mercury (Jernelov, 1972; Vonk and Sijesteijn, 1973). Heuy et al. (1974) reported the transmethylation of stannous and cadmium ions by a mercury resistant bacterium. Other metals may also be methylated by bacteria including the formation of alkyl arsine (Cox and Alexander, 1973), tetramethyl lead (Wong et al., 1975) and dimethyl selenide (Francis et al., 1974).

Physical Factors Affecting Mobilization of Metals from Sediments

Physical factors are involved in the movement of metals from sediment into the overlying water column. Metals are first released into the pore water by one of the chemical processes previously described or by biological processes. The cadmium or other metal may then be moved to the open water by (a) stirring by hydrolic phenomenon, especially in rivers and estuarine tidal areas, (b) disturbances of sediments by benthic macroflora or by gas bubbles from the decay of organic matter, and/or dredging activities (Forstner and Whittman, 1979; Kneip et al., 1974).

Effects of Cadmium on Yeasts and Fungi

Little has been reported concerning the effects of cadmium on yeasts. Saccharomyces cerevisiae could be accommodated to Cd(II) and the accommodation could be induced by Zn(II). When Cd was removed, the yeast underwent a progressive deadaptation. High intracellular levels of Cd in S. cerevisiae may have inhibited divalent cation transport systems (Macara, 1978). Doyle et al. (1975) reported that growth of Aspergillus niger in brain heart infusion was slowed by 10 mg Cd/l and completely inhibited by 80 mg Cd/l. Seven nematode-trapping fungi were studied by Rosenzweig and Pramar (1980) who found that 1 mg Cd(II)/l lowered the growth of four of seven species and that 10 mg Cd(II)/l caused 50% inhibition of mycelial growth of all fungi. Fungi grown on leaves of oak trees which received an aerosol of Cd did not appear to be affected, but pigmented yeasts were sensitive to cadmium (Bewely, 1980). Babich and Stotzky (1977a) reported a sequence of sensitivities to Cd for filamentous fungi and yeasts, but could find no correlation between the class of fungi and sensitivity to cadmium.

Straw columns have been employed with the fungus Pleuroteus cornucopiae Paul ex Fr. to show that Cd could be translocated by biological activity (Brunnert and Zadrazil, 1980). The authors found that (a) translocation was temperature dependent, (b) the age of the columns was of great importance to the translocation patterns generated, and (c) a large percentage of Cd was localized in the fruiting bodies of the fungus.

Effects of Cadmium on Bacteria

Escherichia coli and Cadmium

Cadmium-induced changes in Escherichia coli have been studied mainly

by Mitra and coworkers. Mitra et al. (1975) described changes in E. coli B following exposure to 3×10^{-6} M Cd(II) (0.34 µg/ml). These changes included: development of large intracellular vacuoles, loss of 95% of viability, and delayed initiation of growth. They found that proliferation began without the synthesis of new DNA and that the Zn-metalloenzyme alkaline phosphatase in accommodated (recovered) cells was not inhibited. In unaccommodated cells 2% of the Cd was in the cell walls, 75% was in the membranes, and 23% was in the cytoplasm, while in cells having undergone accommodation, the figures were 56%, 13%, and 31%, respectively. Mitra and Bernstein (1978) showed that the loss of viability observed after treatment with cadmium was accompanied by single-strand breakage in the DNA of the cells. Furthermore, there was a positive correlation between the number of single-strand breaks and the concentration of Cd used. The repair of Cd-induced breaks apparently did not require the presence of DNA polymerase I, although DNA ligase was involved in the repair process (Mitra and Bernstein, 1977). Recently, Khazaeli and Mitra (1981) described the presence of an inducible cadmium-binding protein in E. coli cells accommodated to Cd. The protein had a molecular weight of 39,000 which is much higher than that of mammaliam metallothionein.

Other authors have studied cadmium toxicity by the isolation of a hypersensitve mutant from an E. coli parental strain (Ohta and Udaka, 1977). The mutant was about 1,000 times more sensitive to Cd than the parental strain, being inhibited by 0.5 uM Cd(II) (0.056 ug Cd(II)/ml), and was also sensitive to Zn(II). Reducing agents such as vitamin C, dithiothreitol, and cysteine provided protection from the effects of

Cd(II), while reduced glutathione did not protect the cells from Cd(II). EDTA efficiently reversed toxicity due to Cd(II).

Staphylococcus aureus and Cadmium

Novick and Roth (1968) first described the presence of resistance markers for inorganic ions on penicillinase plasmids of Staphylococcus aureus. The plasmids carried genes conferring resistance to penicillin, erthromycin, arsenate, arsenite, lead, cadmium, mercury, zinc, bismuth, and/or antimony, and the loci for the metal ions were closely linked on the plasmid. There was about a 100-fold increase in Cd resistance of S. aureus harboring the plasmid. Smith and Novick (1972) reported that for three penicillinase plasmids, PI_{524}, PI_{258}, and PII_{147}, there were three separate cadmium loci, cadA - which conferred high-level Cd resistance, cadB - low-level resistance, and mad - which conferred decreased cadmium resistance in plasmid positive strains of S. aureus. Ethidium bromide was used to eliminate Cd-resistance as well as Pb and As which showed them to be closely linked (Kondo et al., 1975). The As, Pb, and Cd linkage group could be transduced into sensitive strains of S. aureus with phage ϕ (Kondo et al., 1975).

Generally, cadmium has been associated with other antibiotics. Most Cd-resistant S. aureus strains isolated in one study were also resistant to penicillin, and many to chloramphenicol, although some which were Cd-resistant were sensitive to all antibiotics (Witte et al., 1980). The same study reported that Cd-resistant strains of S.aureus were found in cattle and man, but none were isolated from sheep, chickens, or pigs.

The mechanism of resistance to cadmium conferred by plasmids in S. aureus has been described as the decreased uptake of Cd by the plasmid

harboring strains as compared to plasmid-negative strains (Chopra, 1971; Kondo et al., 1974; Korkeala, 1979). Oxygen uptake was inhibited by Cd(II), presumably by inactivating respiratory enzymes with thiol (-SH) groups at their active sites (Tynecka and Zylinska, 1974). The inhibitory effect of Cd(II) on S. aureus could be eliminated by CaCl (Kondo et al., 1974) or by cysteine (Tynecka and Zylinska, 1974) in the growth medium. Korkeala (1979) reported that Cd(II) resistance was inducible, and that in both resistant and sensitive strains of S. aureus most cadmium was in the cell wall (70-80%), followed by the membrane (20-30%), and the cytoplasm (~ 2%). Chopra (1971) found that a sensitive plasmid negative strain contained approximately 15 times more Cd than a resistant plasmid-positive strain, and later (1975) reported that protein synthesis in cell-free extracts was inhibited in both strains. The data showed that active metabolism of S. aureus was not required for exclusion of Cd from resistant cells. These results suggest two mechanisms of plasmid induced Cd resistance in S. aureus: (a) conformational changes in the proteins of the cytoplasmic membrane (Chopra, 1975; Kondo et al., 1974), or (b) the ability to distinquish Cd from Ca so that Cd is not brought into the cells (Kondo et al., 1974).

Recent reports suggest that the second mechanism proposed above is not true, but that the first is probably involved in Cd resistance. Tyencka et al., (1981a) have reported that in membrane vesicles of S. aureus Cd(II) uptake was via the Mn(II) transport system. Mn(II) and Cd(II) were shown to be competitive inhibitors of the transport of each other. The plasmid apparently encoded a Cd(II) efflux system which only recognized and removed Cd(II) that was in the cells. This Cd(II) efflux

system was energy dependent and was inhibited by dinitrophenol, N, N -dicyclohexyl-carbodiimide, and incubation at 4°C (Tyencka et al., 1981b).

Cadmium in Other Bacteria

Cadmium-sensitive mutants of Pseudomonas aeruginosa have been isolated (Horitsu and Kato, 1980). The bacterium was also more sensitive to Hg(II) and Cr(II), but not Cu(II). Vacuole-like substances were found in thin sections of the parental strain in the presence of Cd(II), but not in its absence. The sensitive strain took up more Cd than the parent, with a large increase in the percentage of Cd in the insoluble fraction of the cells.

Cadmium-tolerance in Klebsiella (Aerobacter) aerogenes has also been studied (Pickett and Dean, 1976; Pickett et al., 1976). These authors trained K. aerogenes to grow in Cd, i.e., grew the bacterium in increasing levels of Cd in the growth medium (Pickett and Dean, 1976). In media deficient in Mg^{2+}, NH_4^+, or PO_4^{3-}, K. aerogenes was highly sensitive to Cd(II) inhibition (Pickett and Dean, 1976). Pickett et al. (1976) found that the pH-activity profiles of phosphotases differed in resistant and sensitive strains and the level of activity of phosphotases was lower in the Cd(II)-resistant strain. There was a decrease in glucose-6-phosphate dehydrogenase activity, a decrease in glucose phosphoenolpyrurate phosphotransesterase activity, and an increase in phosphoglucose isomerase activity in the Cd-resistant strain (Pickett et al., 1976).

Examination of Cd-sensitive and -resistant strains of Gram-negative bacteria isolated from natural aquatic systems showed that temperature did not affect Cd resistance and that the sensitive strains accumulated more

Cd than resistant strains (Remacle and Houba, 1980). The authors were unable to outline data which explained these observations.

Effects of Cadmium on Bacterial Populations

In the New York Bight, populations of Bacillus sp. resistant to Hg were found to also be resistant to Cd at high levels, and the level was higher in areas of recent pollution with sewage sludge (Timoney et al., 1978). Houba and Remacle (1980) reported that the level of Cd-resistance exhibited was roughly correlated with the degree of contamination in an aquatic system. Most of the bacteria in this study were members of Pseudomonas. Cadmium amendment of a Douglas-fir needle litter microcosm led to bacterial populations enriched for cadmium and gentamycin resistance and streptomycin and chloramphenicol sensitivity (Lighthart, 1979).

Metabolic activities of Cd-treated bacteria in various environments have been investigated. Cadmium was more toxic than As^{3+}, As^{5+}, Zn^{2+}, Cr^{2+}, Fe^{2+}, Mn^{2+}, Pb^{2+}, and Co^{2+} to fermentative activity, but less toxic than Hg^{2+}, Cu^{2+}, Cr^{6+}, Se^{4+}, and Ni^{2+} (Forsberg, 1978a,). Bacteroides succinogenes, Ruminococcus albus, Bacteroides amylophilus, and Eubacterium ruminantium were most susceptable to the metals tested, while Streptococcus bovis was highly resistant. Other investigators have shown that cadmium inhibited nitrification, total glucose metabolism (Mills and Colwell, 1977) and heterotrophic uptake and mineralization of metabolites (Albright et al., 1972) in aquatic systems. Cadmium-treated terrestrial experimental systems showed decreased NH mineralization (Tyler et al., 1974), respiration, and nitrogen-fixation (Lighthart, 1980).

Environmental Factors Affecting Toxicity of Metals to Microorganisms

1. pH. The hydrogen ion concentration (pH) of a system affects metal toxicity toward microorganisms by changing the chemical mobility and form of cadmium anbd other metals. Lower pH increases the solubility of Fe, Mn, Al (Babich and Stotzky, 1978), Ni, Zn (Hutchinson and Collins, 1978), Cd, and Pb (Santillan-Medrano and Jurinak, 1975). At high pH insoluble forms such as hydroxides and carbonates become prevalent (Weber and Possett, 1974). Higher pH caused Pb to be complexed by organics in the medium which became more negatively charged at the higher pH. These Pb complexes were less toxic to Trichoderma vividae than free Pb(II) or hydroxylated Pb (Babich and Stotzky, 1979a). Toxicity of cadmium to Chlorella pyrenoidosa was greater at pH 7 than pH 8 due to increased Cd uptake by cells, probably because larger Cd complexes were formed at pH 8 that could not be easily transported (Hart and Schaife, 1977).

The form of metals present is pH dependent for certain metal species. Babich and Stotzky (1977a) showed that the toxicity of Cd toward bacteria, actinomycetes, and fungi increased with increasing pH. As the pH went from 7 to 9, the amount of divalent Cd(II) decreased as $CdOH^+$ was formed (Hahne and Kroontje, 1973) and the increased toxicity may be due to the greater ability of monovalent cations than divalent cations to penetrate biological membranes (Giese, 1968) which led to larger intracellular Cd levels. Another explanation of the greater Cd toxicity at the higher pH is that there may have been a competitive inhibition effect between H^+ and Cd(II) for sites on the surface of the cells, and as the pH increased the H concentration decreased and more Cd(II) (or $CdOH^+$) sorbed to the cells (Babich and Stotzky, 1980).

Cadmium toxicity to Aspergillus niger increased when soil pH was raised from 5 to 7.2, while the sensitivity of A. fischeri was not pH dependent. Pencillium asperum, P. vermiculatum, A. niger, and Cunninghamella echinulata were more Cd tolerant in natural acidic soil (pH 5.1) than in natural alkaline soil (pH 7.8) (Babich and Stotzky, 1978; 1977c).

The toxicity of Cu(II) and Hg(II) is also related to pH. Fusarium lycopersici was less tolerant to Cu(II) and Hg(II) at increased pH, probably due to reduced competition between H and Cu(II) or Hg(II) for sites on the surface of the fungus (Horsfall, 1956). Chlorella pyrenoidosa was inhibited to a greater extent by Cu(II) at pH 8 than at pH 5, again probably due to less competition between H and Cu(II), and, therefore, more Cu(II) accumulated in the cells (Steemann Nielson and Kamp-Nielsen, 1970).

2. Eh. The oxidation-reduction (redox) potential of an environment is a measure of the availability of electrons and the redox potential (measured as Eh) affects the valence of certain metals such as ferrous and ferric ions. Differently charged metal ionic forms may exert different effects on microorganisms. Cr(III) was not toxic or mutagenic to Salmonella typhimurium, but Cr(VI) was both toxic and mutagenic and caused frameshift mutations as well as base-pair substitutions of the DNA (Petrilli and deFlora, 1977). Cr(VI) decreased rates of fermentation by rumen microbiota by 50%, but Cr(II) was not toxic, and 304 µg As(III)/ml caused a 50% decrease in fermentative activity, while 1,610 µg As(V) was needed for the same effect (Forsberg, 1978a). It has been reported that in Staphyloccocus aureus that there are distinct genetic loci for

resistance to arsenate (As(V)) and arsenite (As(III)) (Novick and Roth, 1968).

The microbial production of S^{2-} from SO_4^{2-} under reducing conditions can cause the formation and precipitation of FeS, CdS, HgS, PbS, and ZnS which lessens toxicity of these metals by removing them from the soluble (available) state (Foy et al., 1978; Krauskopf, 1956). Concentrations of 1 mg Zn(II)/l or 2.5 ug S^{2-}/l inhibited photosynthesis of Selanastrum capricornutum by 50%, but a ratio of Zn:S that resulted in ZnS and no free Zn(II) or S^{2-} in solution had no toxic effects (Hendricks, 1978).

The Eh of a system is also affected by pH. Eh is equivalent to -59 pH (in mV) at 25 C and when the ratio of oxidized to reduced species is 1 (Babich and Stotzky, 1980). Copper disappeared from solution and, therefore, was not available for uptake for microbiota at any Eh value when the pH was greater than 6 or at an Eh less than +200 mV when the pH was less than 6 (Gadd and Griffiths, 1978).

3. Inorganic anionic composition. Divalent metal (M(II)) may form coordination complexes with negatively charged ligands (L) such as Cl^- or OH^- such that $M^{2+} \xrightarrow{L^-} ML^+ \xrightarrow{L^-} ML_2 \xrightarrow{L^-} ML_3^- \xrightarrow{L^-} ML_4^{2-}$ (Babich and Stotzky, 1980). These complexes are generally less toxic to microorganisms than are the free ionic species. Agrobacterium tumefaciens and Aeromonas sp. survived better in sea water with 1 ppm Hg(II) than in lake water with an equivalent Hg(II) concentration, presumably due to the formation and lower toxicity of $HgCl_3^-$/$HgCl_4^{2-}$ complexes formed in the sea water (Babich and Stotzky, 1979b). Divalent Cd(II) was less inhibitory to a mixed sludge microflora than $Cd(CN)_4^{2-}$ (Cenci and Morozzi, 1977), however, Cd(II) and Zn(II) were not toxic to T-even bacteriophage, while

equivalents of CN complexes of either were toxic (Kozloff et al., 1957).

The presence of CO_3^{2-} and PO_4^{3-} may also lead to the formation of insoluble complexes with metal ions. In agar medium, CO_3^{2-} or PO_4^{3-} lowered the toxicity of Pb toward mycelial growth of Aspergillus giganteus and Fusarium solani due to the formation of $PbCo_3$ and $Pb_3(PO_4)_2$ (Babich and Stotzky, 1979a). Aeromonas aerogenes was protected from Cu(II) by PO_4^{3-} by formation of $Cu_3(PO_4)_2$ (MacLeod et al., 1967). Schulze and Brand (1978) showed that Pb toxicity was directly related to PO_4^{3-} concentration in the medium.

4. Inorganic cationic composition. The presence of positively charged ionic species has been shown to affect the toxicity of metals toward microorganisms. Increasing the concentration of K^+ from 0.5 to 3 mM decreased the inhibitory effects of 50 ppb Cu(II) on the photosynthetic capacity of Chlorella pyrenoidosa (Steemann Nielsen et al., 1969). Magnesium ion lessened the toxicity of Ni, Cd, and Co to Aerobacter aerogenes, Escherichia coli, and Torulopis utilis (Abelson and Aldous, 1950) and also reduced the toxicity of Zn(II) (MacLeod and Snell, 1950) and Cd(II) (Laborey and Lavollay, 1973) to Aspergillus niger. However, Mg(II) did not reduce the toxicity of Hg(II) to E. coli (Ohta and Udaka, 1977) or the toxicity of Cd(II) to Staphylococcus aureus (Kondo et al., 1974) or Chlorella pyrenoidosa (Hart and Scaife, 1977). Calcium had no effect on the toxicity of Hg(II) for E. coli (Ohta and Udakaa, 1977) or on Cd toxicity for C. pyrenoidosa (Hart and Scaife, 1977), but lessened Cd toxicity for E. coli (Ohta and Udaka, 1977), S. aureus (Kondo et al., 1974) and A. niger (Laborey and Lavollay, 1973). Growth of Aerobacter aerogenes was inhibited by Hg(II) to a lesser degree when either NH_4^+ or

Mg(II) was present in the medium (Harris, 1958). Ferrous ion (Fe(II)) and ferric ion (Fe(III)) both lessened Cd inhibition of E. coli (Ohta and Udaka, 1977), Fe(III) blocked accumulation of Cd by C. pyrenoidosa (Hart and Scaife, 1977), and neither Fe(II) nor Fe(III) lessened inhibition of E. coli by Hg(II) (Ohta and Udaka, 1977).

5. Clay minerals. Clay minerals are composed of hydrous aluminosilicate compounds and have two important characteristics: (a) a predominant negative charge and (b) a cation exchange capacity (CEC) of about 3 to 5 for kaolinite, 5 to 30 for attapugite, 10 to 30 for illite, 100 to 150 for vermiculite, and 80 to 150 meq/100 g for montmorillonite (Babich and Stotzky, 1978). Heavy metals may be removed from solution by cations on clay minerals, and may also be freed by mass action. Montmorillonite was an effective adsorber of Zn, Cu, Co, Ni, Hg (Krauskopf, 1956), and Cd (Sweeton and Tamura, 1975). Kaolinite was less effective in adsorbing Cd (Anderson and Nilsson, 1974; Gardner, 1974b). The adsorption of Cd by clays from a Cd-Ca suspension follows the order vermiculite > illite > montmorillonite > kaolinite, an order which does not entirely follow the CEC of the clays (Sweeton and Tamura, 1975). Clays protect microbiota from the effects of heavy metals by lower uptake of the metal into the organisms. Protection of bacteria and fungi from the effects of Cd (Babich and Stotzky, 1977b; 1977c) and Pb (Pan et al., 1961) are directly related to the concentration of clay present and to the CEC capacity, such that montmorillonite is more effective than kaolinite.

6. Oganic matter. Organic matter may be humic acids or whole organisms and may protect microorganisms from heavy metal by sorption to particulates, thereby reducing the amount of cadmium or other metal ions

that are available for uptake and hence, toxicity. Cells of Pseudomonas stutzeri have a cation exchange capacity of about 340 meq/100 g dry weight (Zwarm and Thomas, 1973). Uranium was bound by Saccharomyces cerevisiae and P. aeruginosa (Strandberg et al., 1981). Lead was less toxic to various fungal species when humic acids were added to agar, presumably due to binding by the humic acids (Babich and Stotzky, 1979a). EDTA reduced the toxicity of Cu to zoospores of Phytophthora drechsleri (Halsall, 1977), of Cu to E. coli (Milanovich and Wilson, 1975), of Cd and Zn to Klebsiella pneumoniae (Pickett and Dean, 1976), and of Cd to E. coli (Ohta and Udaka, 1977).

The presence of organic contents in media for microbial growth may also affect the toxicity of metals toward microorganisms. Citrate has been shown to chelate Cu(II) and protect Chlorella pyrenoidosa (Steemann Nielsen and Kamp-Nielsen, 1970), to alleviate the toxic effects of Cd(II) and Zn(II) on K. pneumoniae (Pickett and Dean, 1976), and to increase the toxicity of Cd to Pseudomonas sp. due to chelation and co-transport with citrate (Lighthart, 1980). Binding of Cd by thiol containing compounds such as cysteine reduced Cd toxicity to E. coli (Ohta and Udaka, 1977) and S. aureus (Tyencka and Zylinska, 1974). Cysteine has also been shown to reduce Cu toxicity in E. coli (Hirsch, 1961) and Aerobactera aerogenes (Sweeton and Tamura, 1975), Hg toxicity of Aeromonas sp., Agrobacterium tumefaciens and S. aureus bacteriophage 011M15 (Babich and Stotzky, 1979b), and Pb toxicity to Fusarium solani and Aspergillus giganteus (Babich and Stotzky, 1979a). Ramamoorthy and Kushner (1975) reported that yeast extract, Casamino acids, and tryptone were all capable of adsorbing large amounts of Hg, Pb, and Cu (and probably most other metal ions as

well) from solution.

7. Temperature. Toxicity of various metals may be affected by the temperature at which the microorganisms are incubated. Ferrous ion at 1°C had a greater lethal effect on E. coli than at 37°C (Catlin, 1953). S. aureus adsorbed less Cd at 4°C than at 37°C, which led to decreased toxicity at the lower temperature (Kondo et al., 1974). A. niger showed the same level of sensitivity to Zn(II) at 25° and 37°C when no NaCl was present, but in the presence of 0.5 M NaCl the fungus was more sensitive to Zn(II) at 25° than at 37°C.

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